DPhil in Cancer Science

University of Oxford

Clinical

2025 Intake Project Booklet

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DPhil in Cancer Science – Clinical/ Medical Undergraduate Project Booklet

Introduction

This handbook provides an overview for prospective students looking to study for a DPhil in Cancer Science starting in 2025 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

The DPhil in Cancer Science is currently open to Track 2 applicants. Medical undergraduates will **undertake a 3-year research project selected from this advertised 'DPhil in cancer science – Clinical/ Medical Undergraduate booklet'.**

All students are admitted directly to work under the supervision of a Principal Investigator who is formally appointed as the DPhil supervisor.

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).

Degree-level qualifications

As a minimum, applicants should hold or be predicted to achieve the following UK qualifications or their equivalent:

- For medical students not currently at Oxford: successful completion of **at least the first two years of a primary medical qualification (MBBS, MBChB or equivalent)** *and* **a first-class or strong upper second-class undergraduate degree with honours** in a relevant discipline such as biology, biochemistry, or medicine; *or*
- For medical students currently at Oxford: successful completion of the **Pre-clinical Course (First BM)** *and* a **first class or strong upper second-class BA Honours in Medical Sciences**.

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 stipend, University/College fees, and a research consumables budget of ~£13k p.a.

Stipend provisions are summarised below:

- **Application Track 2:** 3 years of stipend at the flat rate of £21,000 per annum.

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 midday, Friday 25th April 2025.** Shortlisted students will be invited to interview in May. It is strongly suggested that students contact supervisors of projects they are interested in applying for prior to application.

Projects

Projects are listed below. Clicking on a project title below will take you to the relevant project page.

Contents

1. A Functional Genomics Approach to Decipher GSC-Macrophage Interactions for Enhanced GBM Therapy – Sneha Anand

Primary Supervisor: Sneha Anand
Additional Supervisors: Daniel Ebner
Eligibility: Track 2 applicants are eligible to apply for this project

Abstract
Glioblastoma (GBM) represents a formidable challenge in oncology, characterized by rapid growth and an immunosuppressive tumor microenvironment (TME). Tumor-associated macrophages and microglia (TAMs) are pivotal components within the GBM TME, driving immunosuppression and tumor progression. Despite promising advances in immunotherapies across various cancers, their efficacy in GBM is hampered by this immunosuppressive milieu. This study aims to elucidate the intricate interplay between patient derived glioma stem cells (GSCs) and TAMs. Through a comprehensive genome-wide CRISPR screen, we seek to identify tumor-intrinsic genes crucial for macrophage-mediated tumor eradication via phagocytosis.

Introduction
Glioblastoma multiforme (GBM) is the most aggressive primary brain tumor, marked by rapid growth and a highly immunosuppressive TME. Standard treatments, including surgical resection followed by chemoradiotherapy, result in a median overall survival of only 14 months. While immune checkpoint inhibitors (ICIs) have improved outcomes in various cancers, they have shown limited efficacy in GBM due to the highly immunosuppressive immune TME composed primarily of microglia and macrophages, collectively known as TAMs. Recent research has identified TAMs as effector cells for tumor cell phagocytosis in response to CD47 blockade, but variability in this response suggests additional unknown signals. Through this study we aim to understand the interaction between glioma stem cells (GSCs) and TAMs using a comprehensive genome-wide CRISPR-Cas9 screen to identify tumor-intrinsic genes essential for macrophage-mediated tumor killing through phagocytosis.

Background
Immunotherapies, including immune checkpoint inhibitors (ICIs), have limited efficacy in glioblastoma (GBM) due to tumor-associated macrophage (TAM)-mediated immunosuppression (Mantovani, A et al, 2017). Macrophages play a dual role, either promoting tumor growth or facilitating its destruction, depending on external signals. A key mechanism is the “don’t eat me” signal, primarily involving the CD47 protein on cancer cells. CD47 binds to signal regulatory protein alpha (SIRPα) on macrophages, inhibiting phagocytosis and enabling cancer cells to evade immune detection (Chao et al. 2012). This signalling is typically present in normal cells but is overexpressed in tumor cells, helping them avoid immune destruction.

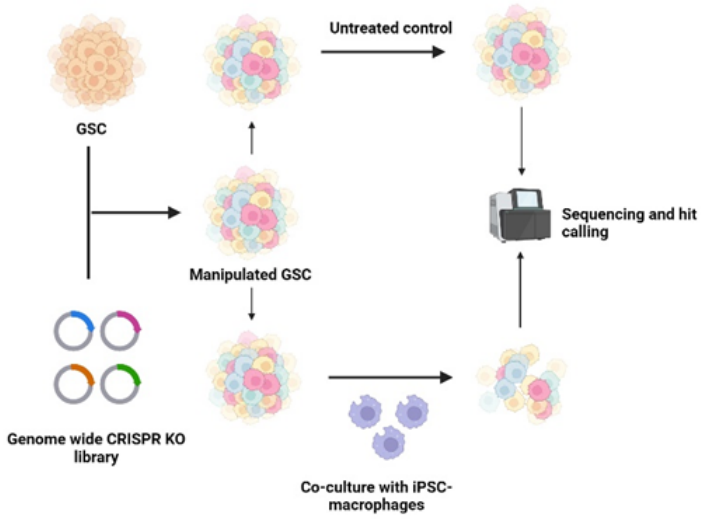
Drugs targeting CD47 can enhance macrophage phagocytosis by blocking the CD47-SIRPα interaction, thereby promoting tumor clearance. Similarly, another “don’t eat me” signal, CD24, interacts with Siglec-10 on macrophages, transmitting inhibitory signals that reduce macrophage phagocytosis (Barkal, A et al 2019). Blocking CD24-Siglec-10 interactions has shown potential in enhancing macrophage-mediated killing of tumor cells in other cancer types.

Despite these insights, the precise dynamics governing GSC-macrophage interactions and the essential tumor-intrinsic features facilitating macrophage-mediated tumor elimination remain obscure. This study proposes a comprehensive genome-wide CRISPR screen to unveil genes within GSCs pivotal for macrophage-mediated killing. Utilizing patient-derived GSCs and iPSC-derived macrophages, we will conduct co-culture experiments coupled with CRISPR-mediated genetic screens to delineate the genetic landscape essential for macrophage-mediated tumor cell clearance.

Methodology

1. **Genetic Manipulation of GSCs and Co-Culture screen with iPSC-Derived Macrophages/Microglia**: Patient-derived GSCs will be transduced with a whole-genome CRISPR-Cas9 knockout library.

   Manipulated GSCs will be co-cultured with iPSC-derived macrophages/microglia to investigate key genetic interactions essential for macrophage-mediated phagocytosis.



2. **Functional Validation**: Identified candidate genes will undergo functional validation through knockout studies to confirm their roles in macrophage-mediated killing of GSCs and their potential as therapeutic targets.

Fig 1: Methodology for CRISPR screening

Expected Outcomes and Significance

This study anticipates uncovering novel tumor-intrinsic genes crucial for macrophage-mediated tumor eradication in glioblastoma (GBM). Functional validation of these genes will provide insights into their roles in GBM progression and their potential as therapeutic targets. By disrupting the immunosuppressive crosstalk between GBM cells and tumor-associated macrophages (TAMs), particularly targeting the "don't eat me" signals like CD47 and CD24, this research holds promise for enhancing the efficacy of existing therapies and offering new avenues for GBM treatment. While monoclonal antibody therapies utilizing antibody-dependent cellular phagocytosis (ADCP) show promise in immunotherapy, challenges persist in GBM due to the low phagocytic activity of macrophages within the tumor microenvironment (TME) and the expression of anti-phagocytic factors by GBM cells. By employing a functional genomic approach to elucidate tumor-macrophage interactions in GBM, this study seeks to identify key tumor proteins involved in these interactions, including both "don't eat me" and "eat me" signals, thereby paving the way for targeted therapies. This research aims to enhance the understanding of macrophage-tumor interactions in GBM and identify potential therapeutic targets, offering transformative implications for GBM treatment.

Training Opportunities

This research project offers valuable training opportunities in CRISPR-based cell screening, cell biology, molecular biology, and bioinformatics analysis. The student will learn essential techniques for gene editing, high-throughput screening, and cellular manipulation. They will gain hands-on experience in cell culture, molecular biology techniques, and an introduction to bioinformatics data analysis. The project will enhance their skills in experimental design, data interpretation, literature review, and scientific writing. Overall, this project provides a solid foundation for their DPhil studies and future research in cancer biology and therapeutics.

Ideal student background: A student with a background in biomedical sciences, cancer biology, or a related field is suitable for this project. The project requires some basic understanding of molecular and cell biology. While it is not expected for the student to have expertise in all areas, a combination of theoretical knowledge and practical experience in the mentioned fields will enable them to contribute effectively to the project's delivery. Additionally, the student should possess critical thinking skills, attention to detail, and the ability to work independently as well as part of a research team. Strong communication and organizational skills are necessary for effective collaboration and project management.

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Return to Projects list

2. Engineering Multicellular 3D Microtumours to Model Ovarian Cancer Minimal Residual Disease – Hagan Bayley

Primary Supervisor: Hagan Bayley
Additional Supervisors: Ahmed Ahmed
Eligibility: Track 2 applicants are eligible to apply for this project

Abstract
Ovarian cancer is one of the most common cancers in women. Drug resistance in cancer treatment is responsible for the majority of cancer deaths. Cancer cells remaining after treatment, termed minimal residual disease (MRD), are capable of reinitiating tumours. Our understanding of MRD biology in solid tumours is limited due to the difficulty associated with isolating and characterising MRD cells from patients. Here we propose to engineer a representative 3D MRD model containing both chemotherapy resistant cancer cells and adipocytes which are known to play an important role in ovarian cancer relapse. The MRD cells will be generated from cancer organoids derived from the biopsies of ovarian cancer patients. We aim to develop a microfluidics-based method to fabricate the 3D microtumours with MRD cells surrounded by adipocytes and compatible extracellular matrix (ECM), mimicking the tumour microenvironment of MRD. We will also test the effect of different ECM materials in MRD progression. The MRD model will be used to investigate molecular pathways involved in the survival of MRD cells and screen novel therapeutics that specifically target MRD.

Research objectives and proposed outcomes
a) Background: Ovarian cancer is one of the most common cancers in women and accounts for around 4,100 deaths each year in UK.[1] Importantly, only 1 in 3 ovarian cancer patients survive over ten years. Around 70% of patients develop recurring cancer after treatment which consists of surgery and adjuvant chemotherapy. The front-line chemotherapy for ovarian cancer consists of cisplatin or carboplatin combined with paclitaxel.[1] Resistance to these drugs is common and leads to cancer relapse and mortality. Residual cancer cells that remain after clinical treatment are called minimal residual disease (MRD). MRD cells share phenotypic and genomic characteristics with the primary bulk tumour, but are capable of reinitiating tumors.[2]
Targeting MRD can prevent cancer relapse and increase the rate of long-term response. For haematological malignancies, personalised treatment of MRD has demonstrated the possibility of achieving long-term cures.[3,4] However, treating MRD in solid tumours is so far largely unexplored. Our current understanding of MRD survival mechanism is limited. Sampling MRD cells from patients with solid tumours is challenging, due to the difficulty in detecting and isolating these cells and also their scarcity. Therefore, there is a great need for building representative MRD models for both mechanistic studies and testing novel treatment strategies.

b) Project plan and previous work: Here we propose to develop a multicellular 3D MRD model derived from patients' cancer cells to i) investigate molecular pathways associated with MRD in ovarian cancer, and ii) screen novel therapeutics to eradicate MRD. We also propose that patterning MRD cells with surrounding adipocytes in Extracellular Matrix (ECM) representative of ovarian cancer will recapitulate the tumour microenvironment, which is important for ovarian cancer relapse.
Previously, the Ahmed lab performed transcriptomics analysis of biopsies from ovarian cancer patients after chemotherapy. We revealed that MRD cells share similar molecular signatures as tumour-initiating cells, expressing adipocyte-like gene signature and dependent on fatty acid oxidation (FAO) for survival and resistance to chemotherapy.[5] Further, the Bayley Lab has established a high-throughput 3D microtumour platform using microfluidics (**Fig. 1A-B**).[6] The 3D microtumours derived from cancer cell lines are able to recreate key tumour features including hypoxia that cannot be achieved using 2D cultures. The drug-resistant 3D MRD microtumours (from cancer cell lines) were able to reflect the non-genetic heterogeneity previously observed in patients' samples (**Fig. 1C**). RNA sequencing revealed that the 3D MRD microtumours resemble MRD in ovarian cancer patients with upregulated genes involved in fatty acid metabolism. We also demonstrated the use of 3D

microtumours for drug development with the identification of a promising FAO inhibitor, perhexiline, that specifically targets MRD cells (**Fig. 1D)**.

To advance and validate the clinic relevance of our 3D MRD microtumour model, as well as develop novel therapeutics against ovarian cancer MRD, we aim to: 1) Generate MRD cells from ovarian cancer organoids derived from patients' biopsies. An ovarian cancer organoids bio-bank derived from patients' primary tumours has previously been established in the Ahmed Lab. The organoids will be treated with chemotherapy drugs to generate the MRD cells. 2) Develop a microfluidic approach to fabricate patterned 3D MRD microtumours with patient-derived MRD cells surrounded by adipocytes, mimicking the MRD microenvironment in vivo. 3) Test the role of different natural ECM materials, including collagen I and hyaluronic acid, in maintaining MRD characteristics. 4) Perform molecular characterisations of the 3D MRD model through live imaging, immunofluorescent staining and RNA sequencing. 5) Test novel therapies. We will test the effect of previously discovered FAO inhibitors, drugs targeting potential new molecular pathways discovered in 4), as well as have co-cultures with T cells to test the hypothesis that drugs targeting metabolism could increase T cell response.[7,8]

We propose that this project will reveal new MRD mechanisms and discover novel treatments. The supervisors on this project have strong track records and will provide important guidance for the potential candidate.

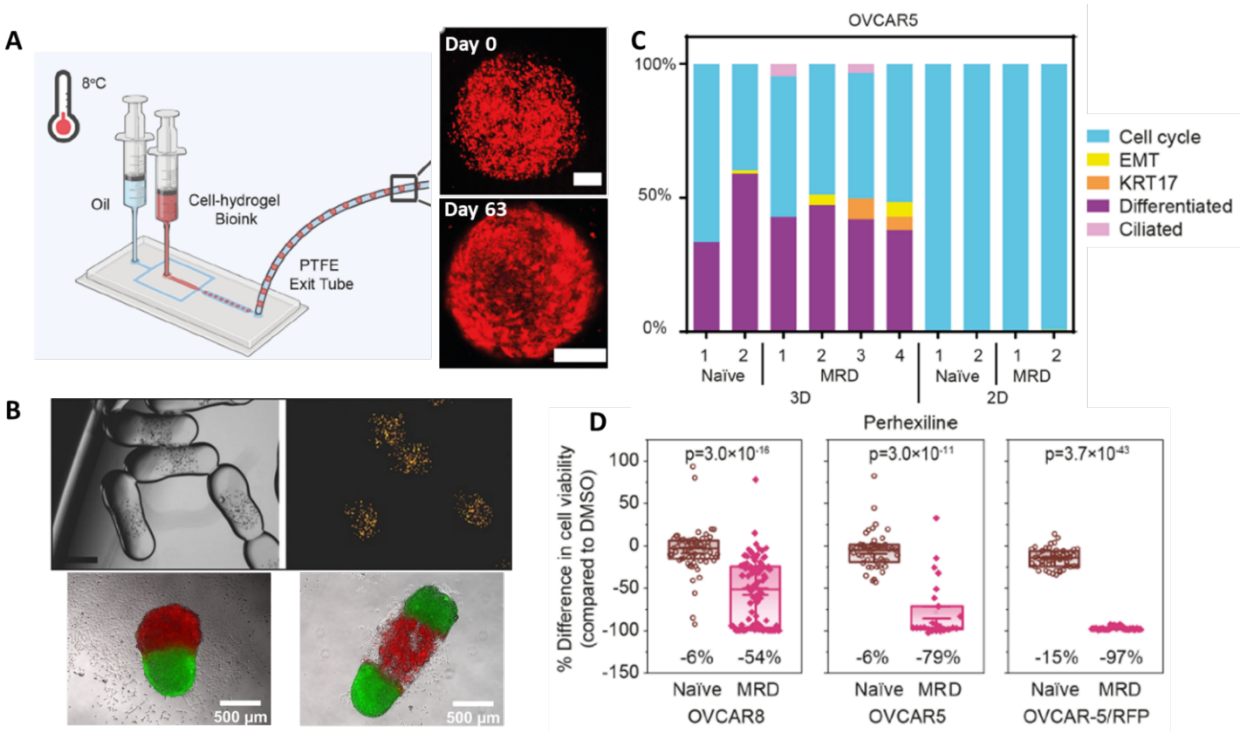

Figure 1: Microfluidic-based 3D microtumour technique and drug testing using the 3D MRD microtumours.[6] **A**, Schematic illustration of 3D microtumour fabrication by the microfluidic platform (left) and long-term culture of 3D microtumours composed of OVCAR-5/RFP (red) and Matrigel (right). Scale bars are 300 µm. **B**, Different patterned 3D microtissues from microfluidics. **C**, Percentage of cells with clinic MRD-related cell states in 3D microtumours and 2D cultures. Data from RNA sequencing result of OVCAR5 cultures treated (MRD) or non-treated (Naïve) with carboplatin. **D**, Perhexiline, a FAO inhibitor, specifically kills MRD cells in 3D microtumours.

Translational potential of the project: Drug resistance is the leading causes of cancer deaths. The proposed project would combine novel microfluidic technique and patient-derived cells to fabricate realistic 3D MRD models that is hard to achieve with organoid method. Specifically, we will advance the previously established

3D MRD microtumour platform by incorporating representative ECM and adipocytes, whose cross-talk with ovarian cancer cells has been widely documented.[9,10] This model would be used for mechanistic studies and drug discovery of MRD. The proposed research aligns with CRUK and the Oxford Centre's research priorities in developing novel therapeutics. New treatment strategies discovered in this project might be used for the treatment of ovarian cancer MRD in clinic.

Training opportunities: The potential DPhil student will be trained in the following fields: 1) Establishment and maintenance of cancer organoids and 3D microtumour cultures; 2) Adipocytes differentiation and their co-cultures with cancer cells; 3) Microfluidic fabrication and hydrogel manipulation; 4) Microscopy (especially confocal microscopy) and general molecular biology techniques; 5) RNA sequencing and data analysis; 6) Testing therapeutics using the 3D MRD microtumours.

Ideal student background: The proposed project is multidisciplinary. The potential candidate will have a background in either cancer biology, bioengineering (or another engineering field), or biochemistry. Experience in cell and organoid culture is desirable. Experience in microfluidics, hydrogels and biofabrication is favourable, but could be learned during the training process. The candidate will work collaboratively with team members engaged in cancer biology, bioengineering and polymer chemistry**.**

References

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3. Applying deep topographic proteomics to solve the problem of ovarian cancer detection – Sarah Blagden

Primary Supervisor: Sarah Blagden
Additional Supervisors: Roman Fischer
Eligibility: Track 2 applicants are eligible to apply for this project

Abstract
Ovarian cancer is the 6th most common cancer in UK and is diagnosed in 7,500 women every year. Like the majority of invasive epithelial cancers, ovarian cancer is preceded by a protracted non-invasive "precancer" phase. For example, high grade serous ovarian cancer (HGSOC) initially develops in the fallopian tube as a preinvasive serous tubal intraepithelial (STIC) lesion and, 4-6 years later, becomes invasive metastatic HGSOC[1]. However, precancers tend to be clinically "invisible" on scans or standard blood tests reflecting their different biological state. For example, whilst the circulating tumour marker CA125 can detect established HGSOC it cannot detect STICs and hence screening using CA125 has proven unsuccessful at improving survival from the disease[2]. Additionally, STICs can only be pathologically detected by microscopy, using paraffin-embedded tissue. This creates a chicken and egg situation: we have no biomarkers to detect STIC lesions pre-operatively, hence cannot remove them as fresh tissue for biological characterisation. However, using state of the art biology, we intend to conduct this research on STIC lesions that have previously been discovered within surgical specimens and archived in paraffin wax.

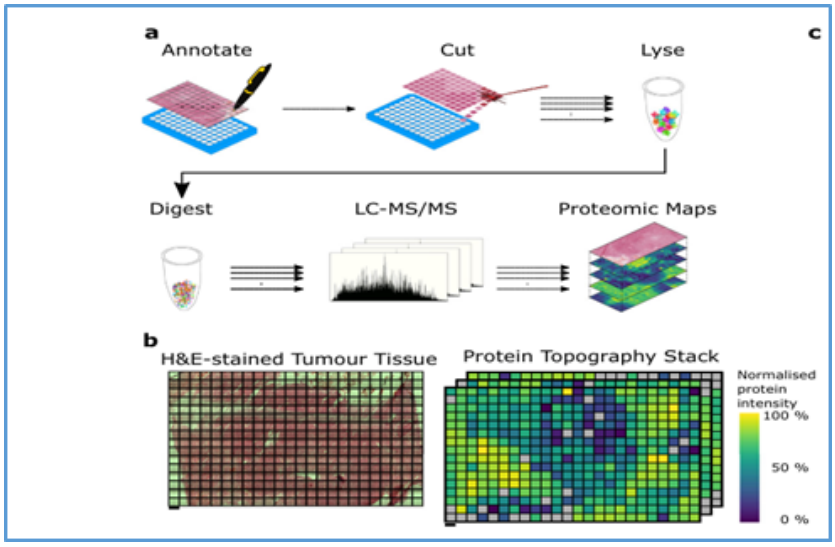
Prof Roman Fischer is the UK's foremost expert in a new technique called LCM-LCMS that combines tumour laser capture microdissection (LCM) with topographic proteomics (LC-MS), enabling detailed characterisation of over 5,000 proteins within individual cells or microscopic regions of tissue. He has optimised this technique to work on paraffin embedded specimens[3]. In this project, we will be using LCM-LCMS to characterise STIC lesions that have previously been resected during other gynaecological procedures (and tend to measure <5mm). LCM-LCMS will provide unbiased information about the proteins within STICs as well as their spatial expression. From this, we will select candidates for further validation as potential ovarian cancer biomarkers

The candidate validation stage will be done in Prof Blagden's lab which has considerable expertise in ovarian cancer research and biomarker development. Using ovarian cell lines and organoids developed in collaboration with Prof Ahmed's prestigious research group, candidate proteins will be selected and characterised using basic molecular techniques such as CRISPR, siRNA and construct-driven re-expression to determine their impact and relevance to tumorigenesis. In parallel, candidates will also be assayed in the circulation of patients with cancer – using immunoprecipitation and ELISA methods initially and then quantified in samples by Parallel reaction monitoring (PRM) mass spectroscopy.

The overarching purpose of this proposal is to characterise STIC precancerous lesions using novel microdissection and topographic proteomics from which new ovarian cancer biomarkers will be identified and validated.

Research Objectives
The project will comprise of four **work packages (WPs).**

WP1. Conduct Laser capture microdissection (LCM) using tissue blocks obtained from ovarian cancer and precancer patients along with adjacent normal tissue for comparison. Conduct liquid chromatography/mass spectroscopy (LC-MS) on excised tissue to identify candidate proteins common across all tissues as well as those unique to precancer.



Overview of the spatially-resolved proteomics workflow in WP1. ***a)*** *Tissue is segmented into a regular grid shape (Annotate), and each element of the grid is isolated by LCM into a well of a 96-well plate (Cut). Proteins from each sample are lysed in RIPA buffer (Lyse) and digested into peptides (Digest) before analysis by LC-MS/MS. The quantitative information for each protein can be mapped back to its location within the gridded tissue and visualised in a topographic protein map, with one map per protein quantified (Proteomic Maps). [figure from ref 1]*

WP2: Validate candidates by IHC using existing tissue microarrays (developed by the Translational Histopathology Lab at ORCRB) containing samples of normal, STIC lesions and HGSOC tissue. Explore the impact of CRISPR-cas9 target depletion in ovarian cancer cell lines and in ovarian organoid models.
WP3: Assay circulating plasma from normal, cancer and precancer patients for levels of identified markers using immune-precipitation/sandwich ELISA. Identify shortlist of candidates to develop PRM mass spec method for detecting constituent peptides in plasma.
WP4: Verify biomarkers in wider set of plasma samples collected longitudinally prior to ovarian cancer diagnosis.

Translational Potential
The earlier detection and prevention of cancer are central objectives of the University of Oxford and CRUK. Using cutting-edge technology, the post-holder will, for the first time, provide unbiased proteomic characterisation of STICs and take the first steps towards biomarker development for this elusive condition. Biomarkers of preinvasive ovarian cancer have enormous clinical potential, not only in detecting it when it is preventable, but also in guiding interventions such as preventive vaccines. If successful, this project could have a major transformative impact on ovarian cancer which is now considered the most lethal of gynaecological malignancy and the techniques developed here can be used to characterise other microscopic precancers.

Training Opportunities
This is an interdisciplinary project between the labs of Prof Sarah Blagden, Prof Ahmed Ahmed and Prof Roman Fischer and offers a wealth of training opportunities. The student will receive training in the cutting, preparation and mapping of specimens for laser microdissection, use of cutting-edge mass spec instruments such as Evosep, timsTOF Ultra 2 and Orbitrap Astral and basic proteomic data analysis. They will learn to maintain cancer cell lines, and organoid models alongside basic wet lab techniques. They will develop insights into biomarker development, clinical approval pathways and a comprehensive understanding of tumorigenesis biology.

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Return to Projects list

4. Understanding the origin of metaplasia – Francesco Boccellato

Primary Supervisor: Francesco Boccellato
Additional Supervisors: Jan Bornschein
Eligibility: Track 2 applicants are eligible to apply for this project

Abstract
Metaplasia is a pathological condition where one type of mature cell is replaced by another type of mature cell that is not normal for that tissue. Metaplasia arise as a result of chronic infections or irritation and it is often considered a pre-cancerous condition. In the stomach, intestinal metaplasia (IM) is epidemiologically associated to chronic *Helicobacter pylori* infection [1] and is the best-known example of metaplasia in the gut. Patients with *Helicobacter pylori* and intestinal metaplasia are at higher risk to develop stomach cancer. Histologically, intestinal metaplasia is recognised by the detection of intestinal specific mucins in stomach histological samples. The normal mucus producing cells of the stomach are replaced with mucus producing cells that are rather typical of the lower gut. Our approach is to reveal signalling pathways involved in this tissue conversion by mapping the expression of single genes and proteins using spatial transcriptomics and proteomics. We will validate these results by pharmacological manipulation of these pathways in primary healthy stomach cells to generate metaplasia in vitro. To this end we are going to use our innovative "Mucosoids" cell culture system, a stem cell drive model which simulates the healthy stomach epithelium in vitro. This research could lead to new diagnostic tests for pre-cancerous conditions and improve early cancer detection strategies, filling a critical gap in understanding the microenvironment of pre-cancerous conditions

Research objectives and proposed outcome
We aim to generate a spatial map of the gene/protein regulatory network that underlie the changes observed in intestinal metaplasia. Our clinical collaborator Dr. Jan Bornschein is involved in the identification of patients with this condition. We will use paraffinized biopsies for spatial transcriptomic or spatial proteomics experiments. We plan map the expression of genes or proteins and we will use algorithms to understand how they interact in different positions of the tissue. The result of this analysis will suggest that specific signalling pathways are regulated differently in specific areas of the metaplasia. We aim to identify the signals triggering those pathways and to use their recombinant versions or pathways inhibitor to obtain metaplastic cells in vitro. For this purpose, we will use our patient derived advanced cell culture called the “Mucosoids”. Mucosoids are a patent pending [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] 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 signals has never been attempted. We aim to find dysregulations in specific 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 is accurate, but have a low sensitivity. A combination of ligands or proteins involved in 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. For track 1 students Clinical training will be provided by Dr. Jan Bornschein. The student will have the opportunity to learn cutting edge technologies such as spatial-transcriptomic, spatial proteomics, 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.

Ideal student background: We seek a student with a strong passion for science. For track 1 and 2 a clinical trainee in Gastroenterology or with an interest in this speciality is required.

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Return to Projects list

5. Equitably implementing improvements in cancer detection – Anna Dowrick

Primary Supervisor: Anna Dowrick
Additional Supervisors: Brian Nicholson
Eligibility: Track 2 applicants are eligible to apply for this project

Abstract
The UK has committed to detecting 75% of cancers at stage I or II by 2028, aiming to deliver this ambitious goal using improved understanding of cancer epidemiology, advances in the development and deployment of diagnostic technologies, and the integration of decision-aids into the consultation and risk-prediction tools into the electronic medical record. Improvements promised by new technologies are rarely equally distributed among populations, and processes of generating data to support new technologies often over-represent majority populations and those who are better educated, healthier and living in urban and affluent areas. There is a risk of perpetuating barriers to diagnosis through implementing tools and technologies of cancer detection that do not reflect the needs of multiple populations. Foregrounding inequalities enables reflection on how the implementation of new initiatives can be designed to deliver greater equity in cancer diagnosis.

Despite a policy commitment to equitable implementation of improvement in cancer detection, there is limited research into the equity considerations of a range of priority cancer detection technologies: i.e. multi-cancer early detection tests (MCEDs), risk-prediction models. For example, it remains unclear how these new detection approaches might differentially impact health outcomes for ethnic minorities, people living in areas of deprivation, and people with learning disabilities. The successful candidate will be supported to develop and lead research into the equitable implementation of new technologies of cancer detection using methods that suit their intended career path.

Research objectives and proposed outcomes.
There is scope for the successful candidate to develop research objectives within the broad framework of CRUK Oxford Cancer Centre's Early Detection theme. The Early Detection theme includes a range of projects which include: evaluating the implementation innovations to improve colorectal cancer detection; optimal pathway design of MCEDs; non-specific symptom pathway optimisation. The successful candidate will be supported to develop and lead research into implementation using methods that suit their intended career path. Examples of areas for development could be to:

- collect primary data in novel implementation research designed by the candidate
- contribute original investigation within existing national implementation projects being conducted by the supervisory team.
- conduct secondary analysis of qualitative data collected from patients and healthcare professionals to explore the equity implications of early cancer detection initiatives
- expand existing implementation research projects to include a deep-dive into the experiences of minoritised groups to inform the optimal development of new cancer detection pathways
- conduct secondary analysis of existing quantitative datasets relevant to the implementation projects to see how they are dealing with race/ethnicity
- explore processes and practice leading to the membership of early detection research cohorts and biobanks to optimise diversity of inclusion

Across all projects the candidate will be get experience of conducting patient and public involvement and engagement (PPIE).

Translational potential of the project
In order for the NHS to both improve rates of cancer detection and reduce inequality in cancer outcomes it is vital to understand the factors that will ensure positive change is implemented equitably. This project will help address the implementation gap, characterising best practice in implementing improvements in cancer detection. Oxford is uniquely placed to investigate equitable implementation of cancer detection technologies as the supervisory team are involved in the development of MCED technologies and risk scores and NHS evaluations of both 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 Cancer Theme majors in implementation science, health records analysis, diagnostic reasoning, and prospective studies of interventions to improve early detection in symptomatic patients. The Medical Statistics Group specialises in quantitative diagnostic, prognostic, monitoring, and prediction methodologies, and the Medical Sociology and Health Experiences Research Group specialises in social science informed, qualitative and mixed methods implementation studies of health and illness, the NIHR Applied Research Collaboration (ARC) Oxford and Thames Valley provides support for applied health and care research that responds to the needs of local populations and health and care systems.

Ideal student background: The doctoral candidate shall have a background in applied health research, social sciences, or a related discipline. Prior experience in working with qualitative data, electronic health records, or similar medical databases is highly desirable. The candidate should possess a keen interest in cancer research, particularly in early cancer detection, and have a passion for health equity. Strong communication and collaboration skills are essential, as the project involves an interdisciplinary team. The candidate should be interested in leveraging cutting-edge techniques and innovative approaches to advance cancer diagnosis. Candidates from minoritized backgrounds are encouraged to apply.

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Return to Projects list

6. Integrating molecular and digital pathology to enhance prediction of breast cancer progression – Kezia Gaitskell

Primary Supervisor: Kezia Gaitskell
Additional Supervisors: Gillian Reeves
Eligibility: Track 2 applicants are eligible to apply for this project

Abstract
Breast cancer is the most common cancer in the UK, and 1 in 7 women will develop breast cancer in their lifetime [1]. Many invasive breast cancers are thought to develop from a precursor lesion, ductal carcinoma in-situ (DCIS), but evidence is limited as to the key factors governing the progression from DCIS to invasive breast cancer. Previous epidemiological analyses in the Million Women Study cohort have investigated potentially-modifiable lifestyle risk factors for DCIS and invasive breast cancer, and suggested that BMI may be a risk factor for disease progression [2]. Other investigators have taken the approach of detailed molecular pathology analyses of a small number of cases, comparing differences in gene expression between DCIS and invasive breast cancer in isolation and in patients with both, in order to identify putative molecular drivers of progression from DCIS to invasive breast cancer [3].

In this project, we aim to triangulate epidemiological data and detailed molecular and digital pathology phenotypes from cases of in-situ and invasive breast cancer in a large prospective cohort study, to enhance our understanding and prediction of the progression from in-situ to invasive breast cancer.
The Million Women Study (MWS) is a prospective cohort of 1.3 million UK women, with detailed prospective information on anthropometric, lifestyle, and reproductive factors [4]. 90,000 cases of breast cancer have accrued after 20 years of follow-up in the cohort overall, including >6000 cases of invasive breast cancer and >800 cases of in-situ breast cancer in the Oxford area.

Germline exome sequencing data from blood samples are also available for a subset of participants in the Million Women Study cohort, including for >12,000 women with invasive breast cancer, >2000 women with DCIS alone, and >500 with both. Of these, approximately 400 women with invasive breast cancer and 60 women with DCIS alone are from the Oxford area, for which tissue samples may be available for molecular and digital pathology analyses.

In this project, exome sequencing data from the sub-cohort will be used to develop a common- and rare-variant genetic prediction model for progression from DCIS to invasive breast cancer. The key germline genetic predictors identified in these analyses will then be taken forward for further molecular testing and validation in the subset of cases with tissue samples in Oxford.

We aim to retrieve archival histopathology slides and tissue blocks from selected cases of in-situ and invasive breast cancer in MWS participants in Oxford, and perform detailed digital and molecular pathology analyses. This will include scanning the glass slides to generate digital whole-slide images, which can be analysed using AI/ machine-learning techniques, and performing molecular characterisation on tissue blocks.
Spatial molecular techniques (e.g. spatial transcriptomics/ proteomics) will be used to investigate whether these key germline genetic variants, identified from exome sequencing analyses, correspond to tissue-specific variation in gene/ protein/ mRNA expression, comparing between normal background breast tissue vs in-situ vs invasive breast cancer. Other molecular differences between in-situ vs invasive breast cancer will also be investigated. Digital pathology image analysis and AI/ machine-learning techniques will also be used to explore imaging-based correlates of these molecular changes. Alternative and/or additional analyses will also be considered and developed in consultation with the student.

This combination of large-scale epidemiological risk factor data on the whole cohort, with rich genomic and digital and molecular pathology data on a subset of cases, will provide an unparalleled resource to investigate the biological mechanisms underlying the progression from in-situ to invasive breast cancer, with important clinical applications for prognostication and planning treatment.

Research objectives:

1. Develop a common- and rare-variant genetic prediction model for progression from DCIS to invasive breast cancer, using exome sequencing data from blood samples from MWS participants with in-situ and invasive breast cancer.
2. Characterise the molecular profiles of selected breast cancers (including invasive, in situ, and those with both in situ and invasive disease present) among MWS participants with archived tumour tissue samples available in Oxford, based on immunohistochemical markers (ER, HER2, PR, Ki67, androgen receptor status), and spatial 'omics' analysis of archival tissue samples, and compare these profiles according to the co-existence of in situ and invasive disease. Explore whether the genetic variants identified from exome sequencing analyses correspond to expression differences in tumour tissue.
3. Validate existing machine learning (ML) based algorithms for prediction of molecular subtype and other molecular biomarkers based on digitised pathology slides in these patients, and compare their performance with that from a novel ML based algorithm derived from these data.
4. Develop a novel algorithm based on information from digital slides, immunohistochemical, and other multi-omic biomarkers for predicting the probability of progression from in-situ to invasive disease, and of death from invasive disease.

Proposed Outcomes:

1. This project will generate a common- and rare-variant genetic prediction model for the progression from DCIS to invasive breast cancer.
2. This project will provide important new information on detailed molecular profiles of both in situ and invasive breast cancers in a relatively representive sample of UK breast cancer patients aged 50 and over, including information on comparatively novel markers such as androgen status, the prevalence and prognostic significance of which is still unclear.
3. The analysis of tumour molecular profiles according to the degree to which invasive disease occurs alongside in situ disease will help identify those in-situ cancer patients who are most likely to progress to invasive disease.
4. The project will enable independent validation of published ML based algorithms for prediction of molecular subtype using digital slides in a large population-based sample of NHS patients.
5. The project will generate and assess the accuracy of a comprehensive algorithm, including immunohistochemical and molecular markers together with digital pathology, for predicting outcome for both in situ and invasive breast cancers
6. The project will help develop important new collaborations between clinical pathologists, epidemiologists, data scientists and engineers, aimed at exploiting existing data from large population-based studies to address major outstanding questions about the potential value of digital pathology for routine subtype classification and predicting disease outcome

Translational potential

This project has a high potential for clinical translation and enhancing patient care. Improved genetic prediction, and characterisation and validation of digital and molecular pathology markers, of progression from in-situ to invasive breast cancer could inform prognostication and shared decision-making on planning treatment – both for identifying patients with a high risk of disease progression, who may require more aggressive treatment, and also for those with a low risk of progression, for whom less-aggressive treatment may be an option.

Training opportunities
As part of this project, the student will receive comprehensive training in epidemiology, statistical analysis of complex linked health records data, analysis of sequencing data, molecular and digital pathology, and machine-learning methods as applied to digital pathology. There will also be opportunities for developing communications skills through presenting results at scientific conferences, writing manuscripts for publication, and contributing to public engagement activities.

Ideal student background: The selected individual for this project should have strong quantitative skills, preferably including familiarity with either epidemiology and statistics and/ or machine-learning methods. Experience of digital pathology would be advantageous but not essential. They will join a diverse team, gaining expertise in epidemiology, statistical modelling, and machine learning, through comprehensive computational training. The role includes opportunities to present findings at international conferences.

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Return to Projects list

7. Understanding variable clinical outcome in paediatric brain tumours – James Grist

Primary Supervisor: James Grist
Additional Supervisors: Ester Hammond
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract
Brain tumours are one of the most common oncological causes of mortality in the adult and paediatric population. Dr Grist has previously developed an AI-based model using Magnetic Resonance Imaging (MRI) that can identify children with tumours that are highly lethal, leading to a much earlier death than would be expected for that type/grade, linked to abnormal blood supply [1]. Interestingly, this model can identify individual patients with lethal tumours that would traditionally be considered 'low risk' (e.g. pilocytic astrocytoma) and vice-versa. **This project will aim to take these results to the next level and validate the model in a prospective study, as well as understand the underlying tumour biology leading to lethal tumours.** If successful, this project could provide novel therapeutic targets, as well as identify those children that may benefit from inclusion in studies with more experimental therapeutic approaches.

Due to the rarity of paediatric tumours, a UK-wide tumour network, the Children's Cancer and Leukaemia Group (CCLG), has been in place for over 25 years and, through the work of Dr Grist and Dr Wilson, Oxford is now a member contributing to this network. Our working hypothesis is that this decreased blood supply leads to a hypoxic state within the tumour, which in turn pushes the cells toward an aggressive phenotype[2].
We would like to offer the exciting opportunity for a DPhil student to drive collaborative study to understand the underlying biology of this hypoxia phenomenon, and to assess whether the blood supply data hold up in a prospective assessment in children across the UK CCLG network. Oxford is a paediatric neuroncology centre and sees most brain tumour cases in the region. In turn, this gives us the opportunity to capture imaging and tissue from a wide range of tumour types and grades to enable the DPhil, we already have ethics in place for the project.

Research objectives and proposed outcomes

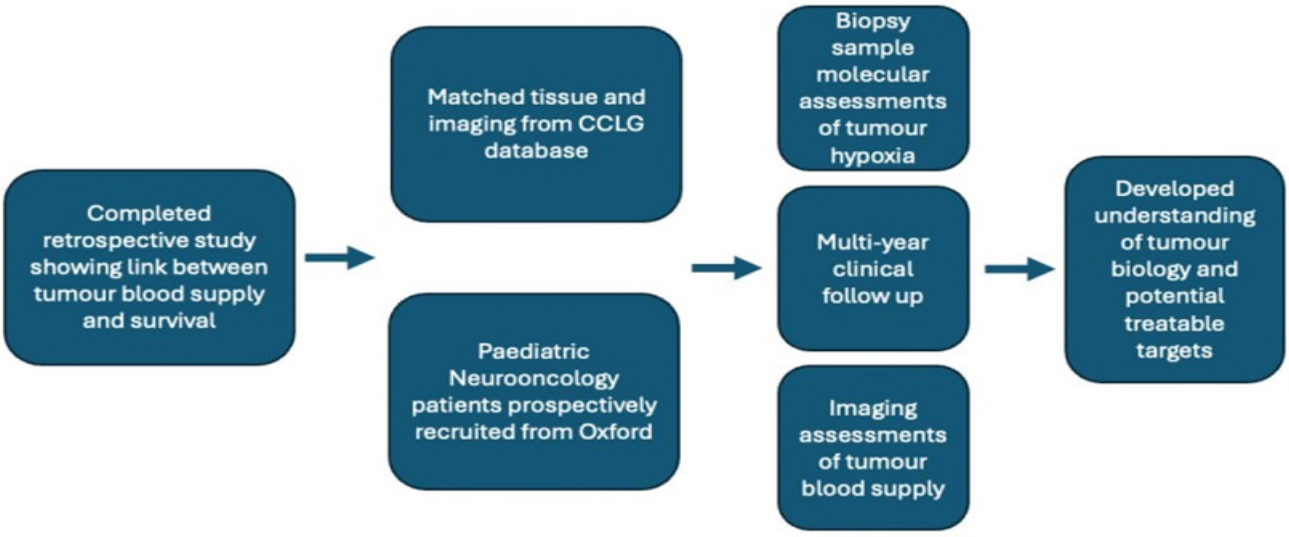

Figure 1 - overview of the DPhil project

The objectives of this DPhil project are:

1) **To assess the imaging-derived tumour blood supply and survival link in a prospective cohort study in Oxfordshire paediatric tumour patients.**

The student will drive a clinical study, in the case of a clinician DPhil they will assist in the consenting of children / parents with a newly diagnosed brain tumour after completing appropriate training and working in tandem with Dr Wilson, ensuring that data is collected from the MRI systems and biopsy samples from surgery. Further data will be collected from collaborating CCLG sites, with matched biopsy tissue from the CCLG tissue bank, to enrich the data set.
The student will, working with Dr James Grist, post-process the imaging data and analyse it using established methods, providing a measure of tumour blood supply to input into the pre-trained machine learning model to assign a 'high risk' or 'low risk' grouping. Participants will be followed throughout the lifetime of the DPhil to assess survival.
Outcome: This work will be publishable in leading journals such as Nature Communications, providing prospective validation of a machine learning model to identify children most at risk of death from a brain tumour.

2) **To derive the underlying biology linking imaging-derived measures of tumour blood supply, tumour vascularity, and hypoxia in biopsy derived samples.**

Whilst imaging data are collected, the student will collect tissue from surgical biopsy and assess it for markers of hypoxia, vascularity, and the underlying genetic regulation of hypoxic pathways, in the Hammond and Olcina labs. We have experience of this imaging/tissue analysis pipeline from the FIG trail that was conducted in Oxford in 2022-2023, assessing hypoxia in adult gliomas. Prospective high- and low-risk groupings derived from the machine-learning model will be used to help stratify data for statistical analysis to assess for differences in hypoxia and vascularity between groups.
Outcome: If successful, and hypoxia is a key difference within and between tumour types, then the student will have developed our understanding of tumour biology, identified potential treatable targets for future therapy development, and validated a potential biomarker for the effect of therapy. Data will be eminently publishable in high-impact journals such as Nature and the New England Journal of Medicine.

Translational potential of the project

This project is highly translational showing the direct link between retrospective clinical data, and a prospective clinical study with underlying biological mechanism elucidated. This is a lab-bench to bedside project with multiple future benefits:

1) A prospective validation of tumour blood supply as a prognostic marker for clinical use.
2) Identification of patients for next generation therapeutic studies
3) Development of our understanding of hypoxia biology and identification of potential treatable targets.

Training opportunities

A) **Scientific skill set development**

Dr Grist will provide the necessary in-depth training for the student to perform MRI imaging acquisition, reconstruction, image registration, post-processing, and analysis. Prof Hammond and Dr Olcina will provide the necessary training in lab-based molecular biology for biopsy sample analysis, as well as the associated quantification and analysis tools of the field. The student will become a multi-skilled professional with a wide-ranging scientific skill set.

B) **Clinical study management**

The student will be mentored by Dr Shaun Wilson, who will seek to develop their understanding of the clinical relevance of their work, as well as the day-to-day challenges of running clinical research studies in the NHS.
This will provide the student with the necessary skill set required to continue their career in clinical research.

Leadership and management

The student will be encouraged to engage with the University training courses in scientific leadership, research integrity, academic writing, and research grant writing. In turn, this will provide the student with the necessary skills to successfully undertake their DPhil and prepare them for the next stages of an academic career.

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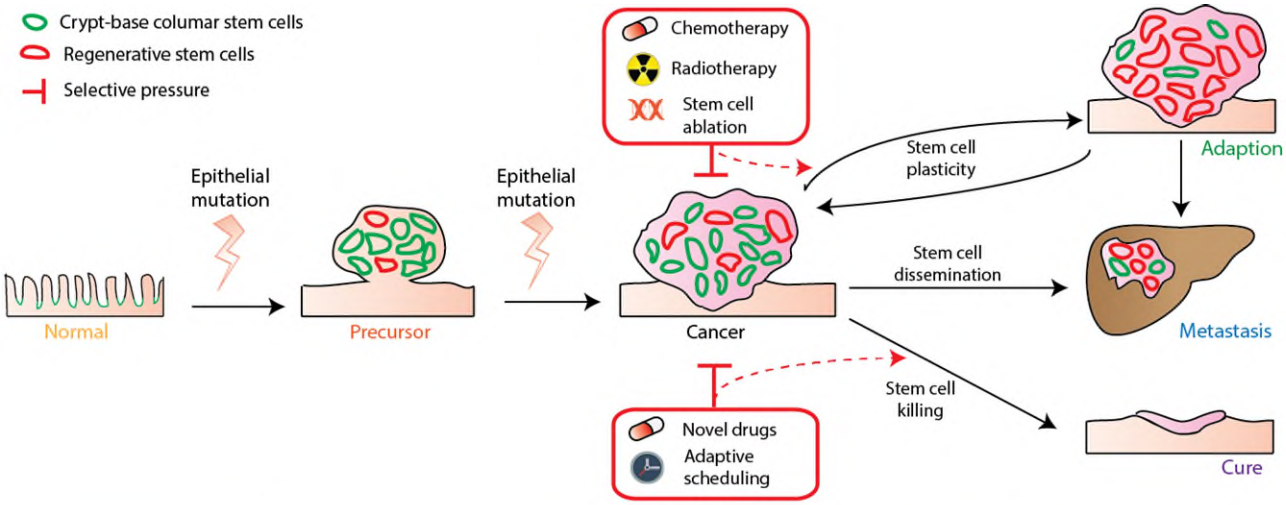
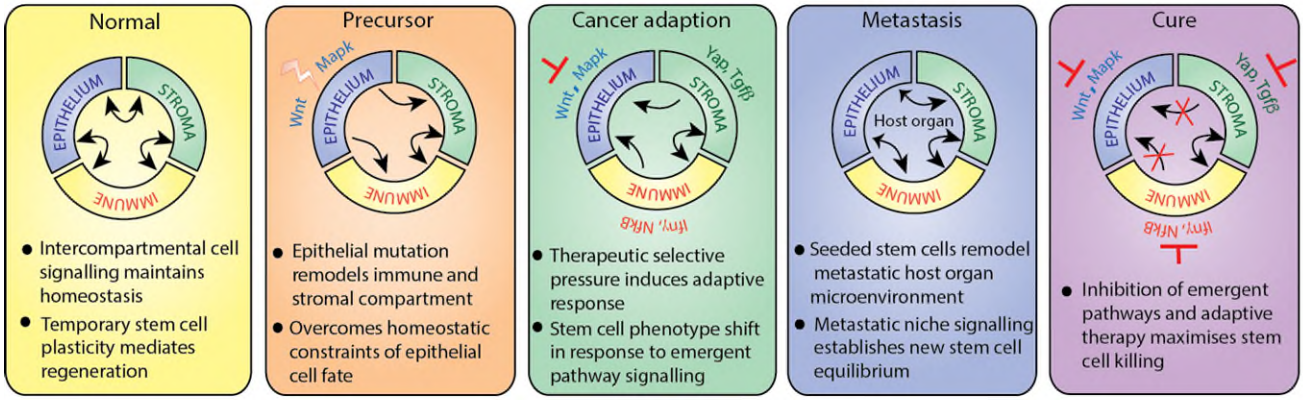
8. Manipulating the stem cell niche to prevent cancer cell adaptive plasticity – Simon Leedham

Primary Supervisor: Simon Leedham
Additional Supervisors: Helen Byrne
Eligibility: Track 2 applicants are eligible to apply for this project

Abstract
Neoplasia is characterised by the activity of stem cells - from the impact of initiating (epi)mutation through to the emergence of therapy resistant clones and metastatic seeding. Stem cell activity is functionally supported by a niche, made up of surrounding matrix, stromal and immune cells. Cancer stem cells are capable of remodelling surrounding microenvironmental landscapes to generate ectopic stem cell niches where bidirectional crosstalk between cellular compartments maintains stem cell phenotype and promotes epithelial adaptive plasticity. Understanding the cell-extrinsic mechanisms driving cancer cell plasticity is key for tackling chemoresistance and developing efficacious new drugs. In this project we will use spatial biology with advanced mathematical analysis to map the cellular constituents of different cancer stem cell niches. We will then generate cancer xenograft models in niche constituent knockout mice to examine the effect of niche modulation on primary and secondary tumour engraftment, progression and capacity to adaptively respond to therapeutic selective pressures.





Background

Neoplasia is characterised by the activity of stem cells - from the impact of initiating (epi)mutation through to the emergence of therapy resistant clones and metastatic seeding. In mouse models, genetic inactivation of the key colorectal cancer driver gene, *Adenomatous Polyposis Coli* (*Apc*) in crypt base columnar cells (CBC's) precipitated rapid tumour induction (*Barker et al, Nature, 2009*). However, other studies subsequently showed that induction of inflammation or disruption of homeostatic morphogen gradients could also lead to neoplasia arising from alternative cell populations outside of the crypt base (termed regenerative stem cells). Our work has shown that colorectal cancers contain multiple populations of stem cells and that these cells are capable of shifting their phenotype (known as cellular plasticity), in response to therapeutic selective pressures, resulting in the rapid evolution of drug resistance (*Gilvasquez et al, Cell Stem Cell, 2022*). Understanding the mechanisms that underpin cellular plasticity and how to prevent it from happening following drug treatments, is key to improving drug response rates and improving patient cancer outcomes.

Research objectives

This project will explore the effect of the tumour microenvironment in mediating cancer stem cell plasticity and seek to mechanistically manipulate key cellular components to attenuate primary and metastatic tumour outgrowth. We will use human tissue and state-of-the art mouse models, deploy spatial biology techniques and analyse the resulting images using existing mathematical methods to assess cellular phenotypes within the tissue context.

Work package 1. Defining the cancer stem cell niche. Stem cell activity - defined by cell self-renewal and multipotency - is dependent on a stem cell niche, which is made up of surrounding stromal and immune cells and matricellular proteins. Cancer stem cells are capable of remodelling surrounding cellular landscapes to generate ectopic stem cell niches where bidirectional crosstalk between cellular compartments maintains stem cell phenotype and functionality, and promotes epithelial adaptive plasticity. Using improving spatial biology techniques (multiplex immunohistochemistry and spatial transcriptomics), capable of identifying epithelial, stromal, immune and matrix markers, we will map different cancer stem cell niches in human and mouse colorectal cancer and liver metastases to identify the cornerstone cell interactions.

Work package 2. Assessing the effect of genetic manipulation of niche cellular components on primary tumour engraftment and progression. Through the CRUK funded ACRCelerate project, we have access to advanced cancer mouse models and organoids across a range of complex mouse genotypes that are disease-positioned to model the human consensus molecular subtypes of CRC. By rectal endoscopic implantation of cancer organoids we can generate orthotopic xenograft mouse cancer models. WP1 will identify genotype-specific primary tumour niche stromal, immune and matrix components. WP2 will establish xenograft models in mice with genetic knockout of key niche components to examine the effect of niche manipulation on tumour engraftment, progression and capacity to respond to therapeutic selective pressures.

Work package 3. Assessing the effect of genetic manipulation of niche cellular components on liver metastasis. Circulating cancer stem cells must remodel the stromal and immune landscape of a distant metastatic host organ, in order to generate a supportive secondary tumour stem cell niche. Our own work has mapped these spatiotemporal events in the formation of liver metastases in advanced mouse models (*Canellas Socias et al, Nature 2022).* In this project, we will use a splenic injection model, allowing temporal control over metastatic cell seeding. We will examine the effect of key metastatic stem cell niche component knockout on secondary tumour engraftment and outgrowth potential, assess mouse genotype specific differences, and use multiplex imaging and maths analysis to look for stromal cell remodelling and innate immune cell infiltration. We will incorporate these data into ongoing development of agent-based models of metastatic outgrowth in a longstanding collaboration with Professor Byrne.

Translational potential

In colorectal cancer (CRC), standard-of-care combination therapies have not significantly advanced in decades, despite huge leaps forward in our understanding of CRC biology. Most treatments target the proliferating cancer epithelium alone, but treatment failure is frequently a consequence of dynamic and multicompartmental tumour adaption to therapy. In order to improve CRC cell targeting we need to understand the microenvironmental pathways that mediate adaptive cellular plasticity, and target them specifically to prevent the evolution of drug resistance. This project will use genetic models to manipulate key stem cell niche components in the hope of identifying new therapeutic targets for the next generation of drug development.

Training opportunities

This project will allow the student to develop their skills with wide and multidisciplinary scientific training. No prior experience is needed. The project will combine wet lab work using mouse models together with spatial biology and deep molecular phenotyping of mouse and human tissue. Students will have the opportunity to learn advanced mouse cancer techniques including endoscopic and splenic xenograft injection. Students will work with mathematical collaborators as the biological input to shared analysis of cellular relationships based on spatial biology dataset interrogation. Students are encouraged to develop their bioinformatic skills to enable them to analyse their own datasets and training for this will be provided. Clinical candidates in Gastroenterology can undertake training in GI family cancer clinics and endoscopy lists.

9. Combining polylipidoid and microneedles for safe and effective intradermal cancer vaccines – Carol Leung

Primary Supervisor: Carol Leung
Additional Supervisors: Molly Stevens and Tim Elliott
Eligibility: Track 2 applicants are eligible to apply for this project

Abstract
Intradermal delivery of mRNA cancer vaccines is a well-known strategy for accessing resident antigen presenting cells to induce tumour-specific cytotoxic T-cell response.[1] Microneedles patches are an emerging alternative to intradermal injections. They offer enhanced patient compliance, consistent and simplified administration, and the potential for increased shelf-stability compared to standard aqueous vaccine formuhations.[2] However, their application is limited by the instability of lipid nanoparticles (LPNs), the clinically approved carrier for mRNA vaccines formulations. Moreover, the immune effects of LNP are incompletely understood, heading to an increased risk of adverse effects. Biodegradable polymers are an effective alternative delivery agent for RNA delivery. For example, poly (CBA- co-4-amino-1-butanol (ABOL)) (pABOL) polyplexes effectively encapsulate and deliver mRNA when injected intramuscularly, and display a short half-life and excellent biocompatibihity.[3] Additionally, lipid-like polymeric constructs (polylipidoids) have been developed which incorporate the advantages of both polymeric and lipid delivery systems. Polylipidoid formulations which readily transfect skin have not been demonstrated. In this project, we aim to develop polylipidoid formulations which effectively transfect dermal dendritic cells and combine them with microneedles intradermal delivery for more effective cancer vaccines.

Research objectives and proposed outcomes

1. **Development and screening of a library of polylipidoid nanoparticles.** Nanoparticles will be fabricated from a combinatorial library of polylipidoid formulations. Such nanoparticles will be characterised and screened for enhanced stability and transfection efficiency in dermal dendritic cells, compared to conventional LPNs formulations.
2. **Development of a microneedle patch for the delivery of polylipidoid-mRNA vaccines.** The best pohyhipidoid-mRNA nanoparticles candidates will be integrated into a microneedle patch for intradermal delivery, with the aim of improving both vaccine shelf-life and administration safety and efficacy.

Evaluation of immunogenicity and efficacy. The immunogenicity and efficacy of the developed vaccine system will be evaluated using both ex vivo human skin explant and appropriate animal models, aiming to demonstrate superior immune activation and cytotoxic T-cell responses compared to conventional LNP systems

Translational potential of the project
The translational potential of this project lies in its ability to significantly enhance cancer vaccine delivery, particularly for intradermal applications. By developing pohyhipidoid formulations that effectively transfect dermal dendritic cells, combined with microneedle patches for intradermal delivery, this project aims to address key limitations of current lipid nanoparticle (LNP)-based systems. This approach promises to improve vaccine stability, simplify administration, and enhance patient compliance. For patients, the benefits include a more effective immune response against cancer, reduced side effects due to better biocompatibility, and the convenience of self-administration through microneedles. Ultimately, this could lead to more accessible and effective cancer immunotherapies, improving patient outcomes and quality of life.

Training opportunities

Within the Stevens group the student will receive training in lipid, polymeric and polylipidoid nanoparticles synthesis and characterisation. Training will include but is not limited to dynamic light scattering (DLS), Single Particle Automated Raman Trapping Analysis (SPARTA), Ribogreen RNA assay for encapsulation efficiency, biocompatibility assays. The student will also receive training in microneedles fabrication and characterisation, including soft lithography and scanning electron microscopy (SEM). The student will also learn how to evaluate transfection efficiency both in vitro and in ex-vivo human skin explants. Within the Elliot's group the student will receive training in a wide variety of techniques including cell culture molecular biology, multiparameter flow cytometry and cellular immunology. In addition, the student will get Home Office Modular training to gain a Procedure Individual License for conducting animal research.

The proposed DPhil project ensures an enriching academic experience within a highly multidisciplinary, collaborative, and translational environment. The student will not only acquire technical proficiency in vaccine formulations, immunology and cancer biology, but also hone transferable skills typical of a doctorate programme, such as ethical research, data management, analysis and interpretation, science communication and many more. By working across disciplines and integrating diverse perspectives, the student will be primed to address complex issues effectively, making substantial contributions to both academia and real-world applications.

Ideal student background: While backgrounds in Pharmaceutical Sciences or Immunology are preferred, this project welcomes individuals from all disciplines who possess a strong passion and inclination for multidisciplinary and collaborative research.

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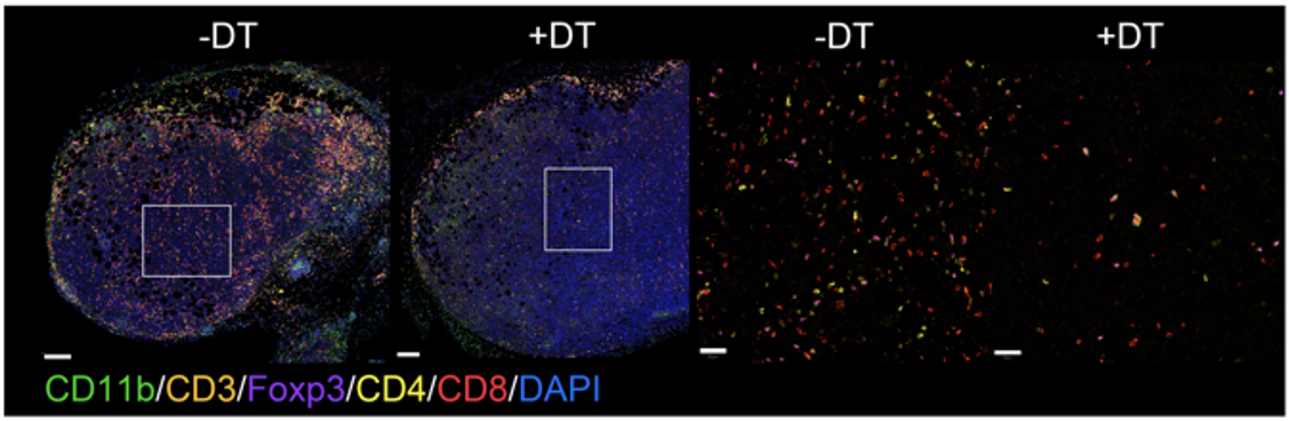
10. Understanding and exploiting cDC1-mediated cross-priming in cancer immunotherapy – Ignacio Melero

Primary Supervisor: Ignacio Melero
Additional Supervisors: Maria Aggleakopoulou
Eligibility: Track 2 applicants are eligible to apply for this project

Abstract
Cancer immunotherapy using immune checkpoint inhibitors (ICI) has revolutionised oncology practice, but fails to show clinical benefit in 70-90% of cancer patients, is not successful for some cancer types, and can have severe side effects[1]. Professional antigen presenting cells (APCs), including dendritic cells (DCs) play an instrumental role in eliciting anti-tumour cytotoxic T lymphocyte (CTL) responses. DCs orchestrate the differentiation and expansion of anti-tumour $CD8^+$ T cell responses via the presentation of tumour-derived antigens to naïve $CD8^+$ T cells, a process called cross-presentation/cross-priming, as well as via the provision of functional cues. Specialised 'conventional type-1 dendritic cells' (cDC1s) have a superior antigen cross-presentation ability to $CD8^+$ T cells and are critical for all known cancer immunotherapies to be effective[2]. Studies by us and others, using cDC1-deficient mice and XCR1-DTR transgenic mice have demonstrated the necessity of cDC1s in effective cancer immunotherapy[3,4,5]. In addition, the presence of cDC1s in the tumour microenvironment (TME) has been positively correlated with the abundance of tumour-infiltrating T cells[6]. We hypothesize that cDC1s offer underexploited opportunities to improve the efficacy and to define biomarkers of effective cancer immunotherapy, therefore the elucidation of the mechanisms regulating their superior antigen cross-presentation ability and function, is of high priority. To explore this, we will use mouse models to determine which tumour neoantigens are cross-presented by cDC1s using advanced proteomics and also mechanistically study the immunological synapse formation between cDC1 and $CD8^+$ T cells.

Figure 1. Depletion of cDC1s in XCR1-DTR mice with diphtheria toxin causes a dramatic reduction of the T cell infiltrate in MC38 engrafted tumours as assessed with a panel of multiplex tissue immunofluorescence

Low magnification High magnification



Research objectives
First, we will apply mass spectrometry-based immunopeptidome profiling approaches[7], established in the Adamopoulou group, to explore the repertoire of MHC-I-bound peptides presented by cDC1 cells within murine tumours and in tumour draining lymph nodes (DLNs). Using already refined immunopeptidome profiling

approaches for small cell numbers isolated from small tissue specimens[7], as well as for isolated and *in vitro* expanded mouse cDC1 cells established in the Melero group, we will profile tumour epitopes that are cross-presented by cDC1 cells. The comparison of the immunopeptidomes derived by murine tumours and tumour DLNs will shed light on the relevance of the location of cDC1s for adequate $CD8^+$ T cell priming. We will use the Colon Tumour 26 (CT26) transfer model, that is a colorectal carcinoma model, where around 85% of the total $CD8^+$ T cell response to 3 epitopes encoded by gp70 have been mapped, or the MC38 colorectal carcinoma transfer model where MC38 cells will be transfected with different sequences of surrogate antigens (i.e. ovalbumin) to interrogate rules of antigen cross-presentation by cDC1s[8]. Novel sFLT-3-based *in vivo* treatments will be applied to augment the numbers of cDC1s in tumour tissues and facilitate the analysis.

Secondly, we will use advanced microscopy and imaging approaches, mass spectrometry and multicolour flow cytometry to characterise the immunological synapse (IS) formation between cDC1s and $CD8^+$ T cells, in collaboration with the Dustin group[9]. These studies will elucidate the help cDC1s provide to $CD8^+$ T cells and the TCR-microvesicles released. This will lead to the identification of receptor-ligand interactions that either enhance or repress the priming or expansion of antigen-specific $CD8^+$ T cell responses following cDC1:$CD8^+$ T cell immunological synapse formation. The role of specific interactions of receptor-ligand pairs, such as CD28-CD80, CD70-CD27, NKG2A-H-2Qa CD137-CD137L, LFA-1/ICAM-1, CD8/MHC-I, IL15-IL15Rα and PD-1/PD-L1 and of paracrine cytokines at immunological synapses of cDC1 and $CD8^+$ T cells are yet not clear and we hypothesize that they can be critical for the outcome of $CD8^+$ T cell cross-priming or expansion. cDC1s are the only physiological source of IL-12[10], among various other costimulatory signals, necessary for the activation of CTLs. These studies will determine whether induction of IL-12 and of costimulatory molecules, such as CD70 or CD137 are sufficient for the activation and/or expansion of CTLs. Furthermore, investigation of the differences of $CD8^+$ T cell priming by intratumour- or tumour DLN-derived cDC1s will offer insight of the interplay of cDC1 cells migrating from the TME to tumour DLNs and the resident cDC1 cells in the tumour, in effective cancer immunotherapy.

Translational potential

These studies aim to shed light on the immunobiology of cDC1 cells and offer new mechanistic insights that could be exploited therapeutically, in combination with cancer immunotherapy. Importantly, data obtained from these studies can set the foundation for clinical trials that investigate cross-priming-based immunotherapies in patients with cancer. Furthermore, the profiling of tumour antigens that are cross-presented by cDC1 cells will improve immunisation strategies that enhance T-cell infiltration into tumours and the monitoring of immune responses upon cancer immunotherapy treatments.

Training opportunities

The DPhil student will be based at the Old Road Campus Research Building. This project provides broad training in cancer immunology, covering a range of cellular and functional immune assays. The student will have access to cutting-edge technologies such as mouse tumour models, cDC1/CD8 co-cultures systems, advanced microscopy, multicolour flow and/or spectral cytometry, mass spectrometry-based immunopeptidomics and comparative bioinformatics on proteomic sequences. The project will enhance their skills in experimental design, data interpretation, literature review, and scientific writing. The student will also have multiple opportunities to present their findings at inter-departmental seminar series and national and international conferences

Ideal student background : You should hold a degree in a relevant discipline such as biology or immunology or related field. A Master's degree in Immunology/ Cancer Immunology will be an advantage but is not a prerequisite. Previous laboratory experience in cellular immunology (e.g. cell culture, flow cytometry, and/or microscopy) will be an advantage but is not required, as the DPhil student will be trained and will have daily supervision as required by senior lab members and collaborators. Excellent communication skills and ability to work as part of a team are essential.

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Return to Projects list

11. Integrating multiparametric MRI with spatial transcriptomics to identify clinically relevant "Radio-Spatial Genomic" features of prostate cancer using artificial intelligence – Ian Mills

Primary Supervisor: Ian Mills
Additional Supervisors: Richard Bryant
Eligibility: Track 2 applicants are eligible to apply for this project

Prostate cancer (PCa) is the most common malignancy amongst men in the United Kingdom [1]. One of the key challenges in the clinical management of PCa involves risk-stratification, to precisely identify the subgroup of men at highest risk of progressing from localised to metastatic disease, and who therefore warrant radical treatment, whilst avoiding overtreatment and associated side-effects for those with lower risk disease who do not need treatment. Multiparametric MRI (mpMRI) has become a cornerstone in the PCa diagnostic pathway, identifying individuals who require a prostate biopsy, and improving the diagnostic biopsy sampling process, whilst reducing unnecessary oversampling. Following pre-biopsy MRI, a prostate biopsy remains an essential next step in the diagnostic pathway to ensure accurate detection of clinically important disease [2], given issues such as moderate inter-reader reliability, false-positive inflammatory lesions and MRI-invisible cancer.

Spatial genomic profiling has emerged as a powerful technique that provides valuable information with which to characterise PCa heterogeneity, and evaluate clonal dynamics, to identify features of potentially aggressive or lethal disease [3,4]. Spatial genomic profiling is currently prohibitively expensive for routine use in clinical practice. However, there is an exciting opportunity to potentially identify important biological features of PCa (from the spatial transcriptomics) within the mpMRI imaging (by amalgamating, or integrating, the clinical parameters, mpMRI radiomics and spatial transcriptomics, into so-called "Radio-spatial genomics"). This novel "Radio-Spatial Genomics" platform has to date not been undertaken for PCa, and therefore offers an exciting opportunity to identify mpMRI radiomic features associated with important biological aspects of PCa linked to an aggressive disease phenotype based on parallel investigation of spatially resolved transcriptomics from corresponding tissue samples. As pre-biopsy mpMRI is now routinely used in the diagnostic pathway for PCa, leveraging the potential wealth of information provided by mpMRI radiomics, much of which is not routinely used in image reporting (such as quantitative features, including textural, grayscale and shape features), may offer additional information beyond that currently available to better risk-stratify patients.

The research team in Oxford has expertise in high-throughput spatial transcriptomics biology, with this technique having been performed in samples from 10 patients with PCa who underwent radical prostatectomy (surgical removal of the prostate), and who have pre-operative mpMRI imaging for analysis. The DPhil student will work within the Nuffield Department of Surgical Sciences, in collaboration with the medical image analysis group in the Big Data Institute, and in collaboration with Urology and Radiology colleagues, to develop and refine a mpMRI and spatial transcriptomics co-registration workflow, to include the use of artificial intelligence (AI)/deep learning. This will involve establishing a novel pipeline to infer the biopsy of "clinically significant"/high risk PCa from the mpMRI images, using an integrated analysis of the spatial transcriptomics, using an integrated analysis approach of paired mpMRI and spatial biology datasets from these highly annotated and well-curated cases. In the longer term, this "Radio- Spatial Genomics" technique may be utilised to infer important biological features associated with an aggressive clinically relevant PCa phenotype from the mpMRI radiology imaging, to improve risk stratification by incorporating

novel radiological features from the diagnostic mpMRI imaging.

Research objectives

- To develop and validate mpMRI and spatial transcriptomics histology co-registration. To achieve this first objective, a convolutional neural network for co-registration will be evaluated using an existing resource of multi-section spatial transcriptomics and pre-operative mpMRI available from a small well-annotated patient cohort. **Collaborators**: Dr MacPherson (Consultant Radiologist), Dr Grist (MRI physicist), Associate Professor Papiez (Medical Image Analysis expert), Dr Colling (Consultant Histopathologist), Dr Figiel (Postdoctoral Scientist), Prof Mills (PCa Researcher), Prof Woodcock (Data Scientist), Prof Lamb (Ca Researcher and Urologist), Prof Bryant (PCa Researcher and Urologist).
- To identify mpMRI radiomic features that correlate with biologically important transcriptomic signatures from spatial transcriptomic analyses, potentially associated with adverse clinical outcomes. Having generated an integrated "Radio-Spatial Genomics" resource in Objective 1), this model will be used to infer important biological features of PCa from the Spatial Transcriptomic data to the Radiological data. This approach will be used to identify radiomic features from genomic features, with these being a potential surrogate for clinical outcomes. **Collaborators**: Dr MacPherson (Consultant Radiologist), Dr Grist (MRI physicist), Associate Professor Papiez (Medical Image Analysis expert), Dr Colling (Consultant Histopathologist), Dr Figiel (Postdoctoral Scientist), Prof Mills (PCa Researcher), Prof Woodcock (Data Scientist), Prof Lamb (PCa Researcher and Urologist), and Prof Bryant (PCa Researcher and Urologist).

Translational potential

This project may lead to evaluation of radiomic features in a cohort of men with pre-operative mpMRI and longer-term follow-up data to correlate inferred "Radio-Spatial Genomics" with phenotype. These features may be evaluated as an adjunct to improve non-invasive risk-stratification for patients with newly diagnosed PCa.

Training opportunities

The student will be provided with medical image analysis training and application of a convolutional neural network during attachment at the Big Data Institute under the supervision of Prof Papiez. Training will be provided by Dr Grist on mpMRI physics and quantitative feature extraction. Dr MacPherson will provide training in clinical image analysis and interpretation, and Dr Colling will provide training in histology and will work with Prof Mills, Prof Woodcock and Dr Figiel to evaluate transcriptomic signatures. Prof Lamb and Prof Bryant will provide guidance on clinical translation and overall direction of the research project.

Ideal student background: Successful applicants should ideally have a background in translational and clinical research, involving patient data

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12. Multi-cancer early detection testing in clinical practice – Brian Nicholson

Primary Supervisor: Brian Nicholson
Additional Supervisors: Eva Morris
Eligibility: Track 2 applicants are eligible to apply for this project

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 and proposed outcomes.
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 of the project
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 Health Research Centre works upstream and downstream of the CE-marking process to both influence the development of novel technologies and the evaluation of clinic-ready products.

Ideal student background: The necessary skillset of the student will be determined by the area of study. For example, a clinical trainee or health services researcher could contribute to clinical implementation and pathway development, a non-clinical scientist to biobanking and analysis of samples, and an aspiring statistician or epidemiologist could develop the models to select patients for MCED testing.

13. Epigenetic control of cancer cell phenotypes via nuclear F-actin based chromosome motility – Eric O'Neill

Primary Supervisor: Eric O'Neill
Additional Supervisors: Yang Shi
Eligibility: Track 2 applicants are eligible to apply for this project

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 differentiation[6]. 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 the mechanical environment and extra cellular matrix influences nuclear actin filaments to influence cell phenotype. We will explore how nuclear actin influences the stabilisation of cell phenotypes through mobilising chromatin and phase separated boundaries[7] and impacts tumour progression from benign lesions in pancreatic cancer.

Research objectives and proposed outcomes
(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 of the project.
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[8]. 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[9], 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[10], 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.

Ideal student background: Capability in cell and molecular biology techniques is preferential but not essential. Prior experience in microscopy would be an advantage but training can be provided.

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14. Exploring the role of Oncostatin M in the stromal-epithelial cross talk during inflammatory bowel disease and tumorigenesis – Fiona Powrie

Primary Supervisor: Fiona Powrie
Additional Supervisors: Mathilde Pohin
Eligibility: Track 2 applicants are eligible to apply for this project

Abstract

IBD is a chronic inflammatory disease of the gastrointestinal tract characterised by a breakdown of the epithelial barrier leading to ulceration and tissue injury. Patients with IBD have a higher risk of CRC, which increases with the duration, extent, and degree of inflammation. Upon tissue injury, epithelial cell repair requires the activation of a "wound-associated epithelial" (WAE) gene program induced by signals from the lamina propria amongst which stromal cells exert a critical role [1]. In IBD tissue with ulceration (wound that does not heal), we have shown that fibroblasts are actively remodelled by the inflammatory landscape and in particular by neutrophil derived cytokines such as Oncostatin M (OSM) [2,3]. Interestingly, this inflammatory fibroblast (FAP+ PDPN+) program is also found in CRC patients alongside neutrophils infiltrates (MPO) suggesting that mechanisms of wound healing in IBD and in CRC are overlapping (Figure 1A). The concept of molecular similarities between tumors and wounds and the description of carcinogenesis as " a wound that never heals" has been in the literature for decades [4] and the WAE gene program is enriched in CRC reflecting the presence of stromal signals promoting epithelium repair [5]. OSM receptor (OSMR) is specifically expressed by stromal cells in the intestine and we have previously shown that targeting OSM signalling in the Helicobacter hepaticus (Hh) + antIL10R mouse colitis model featuring hyperproliferation of the epithelium is beneficial (West et al). Moreover, our preliminary work shows that *OSM* expression is higher in tumour than in the inflamed adjacent tissue of a colitis associated cancer mouse model (Hh+ Azoxymethane AOm) (Figure 1B). We hypothesise

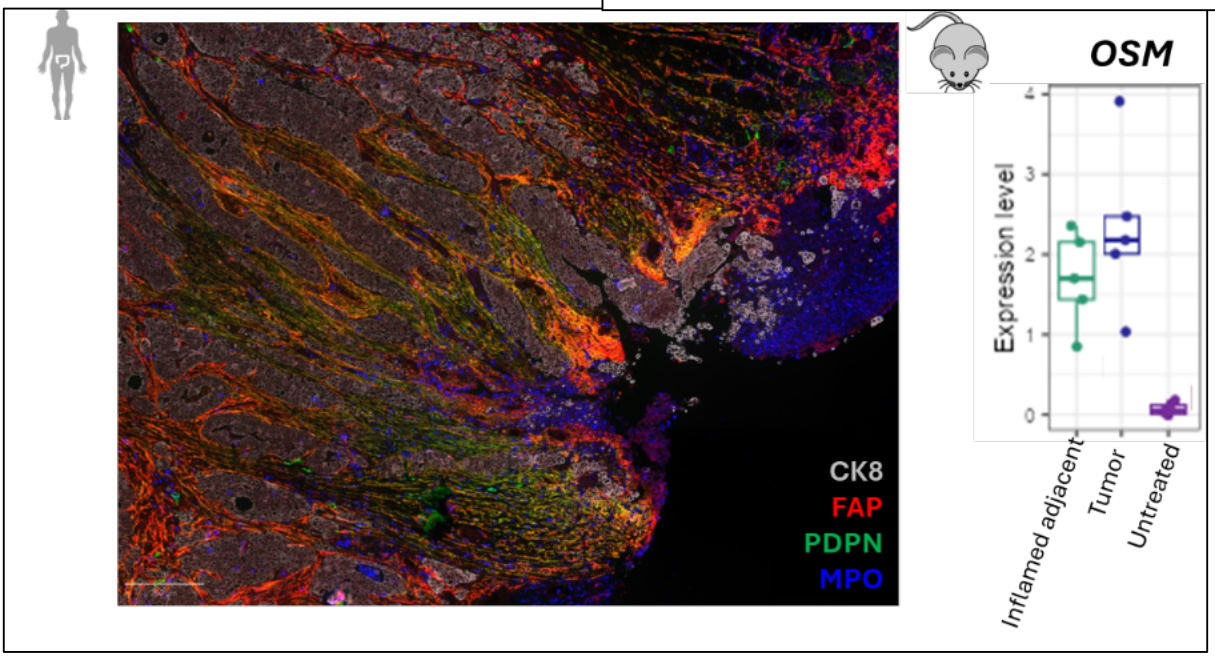

Figure 1. A. Immunohistostaining of resected human CRC tissue showing the presence of neutrophils (Myeloperoxydase MPO) and inflammatory fibroblasts (Podoplanin PDPN, Fibroblast activated Protein FAP) in the tumour (Cytokeratin CK8). B. Transcriptomic expression of *OSM* in the inflamed tissue, tumour distal colon of a HhAOM colitis associated cancer mouse model.

that OSM is required for healing of the epithelium which is impaired in IBD but overactive in CRC. As such, OSM function may be detrimental in CRC. Using our mouse models of colitis, wound healing and cancer, we will characterise the role of OSM in the stromal – epithelium cross talk to understand its contribution to IBD and CRC pathology.

Research objectives and proposed outcomes

Aim 1. Determining the function of Oncostatin M in intestinal wound healing and in colitis. We will use an intestinal biopsy punch would healing model and a microbe-driven colitis mouse models to determine the contribution of OSM to epithelial repair. To this end, we will either overexpress OSM (colonic injection or hydrodynamic injection of recombinant OSM adenovirus) or inhibit OSM expression (blocking antibody and Knock out mice) and characterise the wound healing response using histology, spatial transcriptomic and immunohistochemistry.

Aim 2. Targeting Stromal OSMR expression in colitis associated cancer. We will first characterise the expression of OSM and its downstream signalling gene signature in bulk RNA and single cell RNA sequencing CRC data sets (in house and publicly available) as well as characterising the presence of neutrophils and stromal cells subsets in our own banked tissue of human CRC. The presence of OSM signalling and its associated immune-stromal network will be correlated with prognosis, mutations and CRC subsets. In addition, in vitro human organoid culture and primary stromal cells lines will be used to determine the function of OSM on CRC derived cell types. Finally, we will test the contribution of OSMR signalling (OSMRflox and STAT3flox) in cancer initiation and progression by targeting stromal cells using Platelet Derived Growth Factor (PDGFR)a and PDGFRb CRE^{ERT2} mouse strains and our models of colitis associated cancer induced by Dextran Sulfate Sodium and HhAOM.

Training opportunities

The successful applicant will benefit from a multidisciplinary supervisory team with access to cutting edge multiplex imaging and spatial transcriptomics platforms. 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 clinical samples. This project provides a broad training in cancer biology and immunology covering a range of cellular, molecular, and computational techniques. Students have access to cutting edge technologies such as disease positioned mouse models, *ex vivo* organoid models derived from human patients – co cultures systems.

Ideal student background: We are looking for a highly motivated and intellectually curious candidate with an interest in the intestinal tissue biology, epithelial regeneration, immune-oncology and translational medical research. The successful candidate is expected to be capable of working both independently and in teams, to have good communication skills and possess a general knowledge of the science supporting this project. They will also have had experience in a wet-based lab throughout their studies.

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15. Spatial transcriptomic analysis of the bone marrow landscape in blood cancer – Daniel Royston

Primary Supervisor: Daniel Royston
Additional Supervisors: Ros Cooper and Jens Rittscher
Eligibility: Track 2 applicants are eligible to apply for this project

Abstract
Myeloproliferative neoplasms (MPN) are blood cancers characterised by overproduction of blood cells driven by well-defined driver mutations in *JAK2, CALR* or *MPL.* MPN patients are at increased risk of thromboembolic events with some patients also developing bone marrow scarring (myelofibrosis) or transformation to acute leukaemia, events associated with poor prognosis. Despite recent advances in our understanding of the common molecular abnormalities underlying MPN, it remains unclear why patients with similar driver mutations and clinical characteristics at diagnosis follow divergent disease trajectories. However, it is increasingly appreciated that perturbations in the bone marrow microenvironment and subsequent interactions with neoplastic haematopoietic stem cells (HSCs) are critical for disease initiation in MPN and influence disease and progression. Relatively little is known about the specific spatiotemporal relationships between these cell populations in the marrow of MPN patients. We have therefore developed and refined in-situ spatial transcriptomic (ST) approaches with particular focus on bone marrow tissue sampled from blood cancer patients. This allows high resolution detection and characterisation (phenotypic and genotypic) of individual stromal, immune and haematopoietic cell populations in the marrow of intact tissue biopsies. We will refine and develop computational / bioinformatic approaches to analyse and integrate this ST data, and integrate with recently developed image analysis / AI-powered tools designed to interrogate the morphological characteristics of the bone marrow in health and disease. Outputs from this work will be cross-validated using protein-based approaches including multiplex immunofluorescence and immunohistochemistry across larger clinical cohorts. This project aims to identify markers of early-stage disease progression in MPN suitable for translation to the clinic. It will also support ongoing efforts to validate and inform the search for novel therapeutic targets in MPN and related blood cancers.

Research objectives and proposed outcomes
RO1 - Extend and refine recent pilot ST analysis of bone marrow trephine (BMT) samples in MPN

- Employ recently developed strategies for handling and processing BMT specimens to expand and enrich the latest pilot ST data to create comprehensive whole sample 'single-cell' annotations from locally derived MPN patient cohort
- Apply and develop advanced computational / bioinformatic approaches to quantitatively capture key features distinguishing normal and diseased marrow microenvironment in MPN. These annotations will be validated against both conventional morphology and immunophenotyping.
- Integrate ST findings with those of established bone marrow stromal and immune MPN scRNA-seq datasets from collaborating groups to provide comprehensive annotations of spatially resolved single cell transcriptomic data.

RO2 - Develop computational methods to integrate the ST findings from RO1 with complementary image-analysis based descriptions of established pathological features in MPN

- Utilise our recently developed AI-based megakaryocyte and fibrosis detection and quantitation approaches to characterise associated ST-derived cell microenvironment signatures developed under RO1

RO3 - Establish and support new H+E-based algorithms trained on the integrated ST-derived morphological features (e.g. megakaryocyte and fibrosis) established under RO2 and refine existing AI-based algorithms to support improved diagnosis in MPN.

- Compare the cell signatures of early and advanced morphological abnormalities across important MPN disease subtypes and develop computational models to cluster key cellular and stromal features that are shared or restricted to MPN subtypes
- Train and validate H+E-based algorithms to identify and screen for morphological 'signatures' of early fibrosis and those predictive of disease progression using archival MPN patient samples, with line of sight to large scale clinical validation.

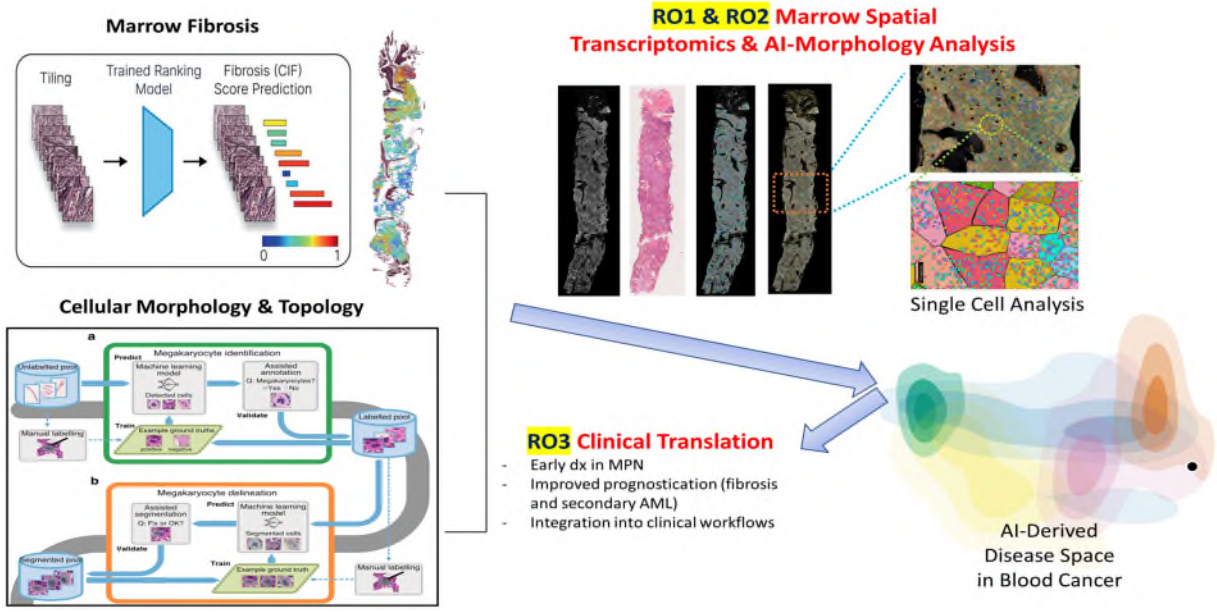

Figure 1. Overview of project workflow

Translational potential

The motivation behind this project is to address areas of unmet clinical need in the assessment of MPN patients, with focus on improving the accuracy and consistency of bone marrow biopsy interpretation and systematically characterising pathological features linked to disease progression in MPN. This work will strengthen and extend our group's commitment to develop personalised diagnostics in blood cancer, with line of sight to the development of validated algorithms suitable for integration into routine clinical reporting. The experimental design and objectives align with the goals of blood cancer patient advocacy groups and established funding bodies with whom we have ongoing support, including Blood Cancer UK, Cancer Research UK, MPN Voice and the MPN Research Foundation. To ensure transparency and maximise the clinical relevance of our work, members of our group regularly contribute to patient and public involvement (PPI) activities coordinated via the Oxford Blood Group.

Training Opportunities

In addition to the generic training opportunities offered by the Oxford Cancer Centre, DPhil students will be trained in a wide range of tissue diagnostic and analytical techniques including conventional microscopy, immunohistochemistry (IHC) / immunofluorescence (IF) microscopy, and spatial transcriptomics. They will also be trained in the analysis of 'omic' data including single cell and bulk transcriptomic datasets. This will involve supervised training in the use of specialist software and incorporate methodologies designed to analyse and

integrate multi omic data from patient samples. Training will be supported by collaborators spanning multiple research themes and clinical / academic departments within the University and NHS. Successful applicants will also participate fully in Prof. Rittscher's successful student training programme at the IBME, incorporating weekly lab meetings.

Ideal student background: The project would suit a clinical or science graduate with a background in computational biology or bioinformatics and big data. Familiarity with the principles of digital image analysis / AI would be desirable but is not essential.

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16. Restricting the emergence of drug resistance in prostate cancer: injectable polymeric microparticles for the localised and sustained release of androgen receptor antagonists – Molly Stevens

Primary Supervisor: Molly Stevens
Additional Supervisors: Ian Mills
Eligibility: Track 2 applicants are eligible to apply for this project

Abstract.
In the UK, about 1 in 8 men will get diagnosed with prostate cancer in their lifetime. Prostate cancer is a hormone-dependent malignancy where the growth and survival of cancer cells are driven primarily by androgens. As such, androgen receptor (AR) antagonists are a cornerstone of prostate cancer treatment. Clinical trials, such as STAMPEDE, have shown significant survival benefit when AR antagonists are administered in the earlier stages of the disease.[1] However, the use of AR antagonists is associated with the emergence of drug-resistant phenotypes and significant systemic side effect.[2] In this project, we aim to develop locally injectable and biodegradable microparticles for the sustained and localized delivery of commonly used androgen receptor antagonists. We will assess the impact of this drug delivery strategy on the development of drug-resistant phenotypes, as well as its safety and efficacy, using pre-clinical cancer models.

Research Objectives and proposed outcomes

1. Development of the drug delivery system. Within the Stevens Group, we are developing a novel drug delivery system using microparticles made from clinically approved and generally recognised as safe (GRAS) surface-eroding biodegradable polymers.[3] Unlike bulk-erodible polymers, like PLGA, these ensure a steady drug release over time. The drug-loaded microparticles are produced via microfluidic-assisted emulsion, offering a scalable and modular platform for fabricating highly monodisperse and reproducible microparticles. The degradation rate can be precisely tuned by adjusting the polymer composition, crosslinking degree, and microparticle dimensions. Preliminary data show that small hydrophobic drugs, similar in structure to clinically approved AR antagonists, distribute uniformly within the polymer matrix during fabrication and are released with quasi-zero-order kinetics. The degradation time can be tailored between 3 and 24 months. We aim to optimise drug loading and degradation profile for the localised, sustained and controlled release of AR antagonists, so to maintain therapeutic levels over the desired period. This approach aims to enhance therapeutic efficacy while reducing systemic side effects and the emergence of drug-resistant phenotypes.

2. Evaluate the impact of the proposed drug delivery system on the emergence of drug-resistant phenotypes in pre-clinical prostate cancer models. Professor Mills' team has notable expertise in constructing pre-clinical prostate cancer models and utilising them to unravel cancer progression, particularly focussing on the emergence of drug-resistance phenotypes such as neuroendocrine cancer cells. These transition states can be identified by gene signatures associated with the activation of transcription factors that drive lineage state change.[4,5] We will use chromatin immunoprecipitation and sequencing and transcriptomics to compare the effect of different drug delivery strategies on the emergence of the neuroendocrine drug-resistant phenotype in a range of pre-clinical prostate cancer models, including prostate cancer cell-lines, patient-derived organoid and *in vivo* syngeneic mouse models representative of both the hormone-sensitive and hormone-insensitive stage of the disease. We will compare our proposed delivery strategy to both conventional AR-antagonists delivery and bipolar androgen deprivation therapy – where patients receive AR-antagonists and high-dose testosterone in alternating treatment cycles - currently in clinical trial.

3. Evaluate safety and efficacy of the proposed drug delivery system in pre-clinical prostate cancer models. Responses in *in vitro* models will be assessed using viability assays and cell death assays to define

short- and long-term cytotoxicity and dose responses. Having defined sub-toxic long-term dose ranges, we will assess efficacy in these conditions based on the suppression of androgen receptor activity (transcriptionally and proteomically). Secondarily we evaluate the emergence of neuroendocrine phenotype, by assessing transcript and protein changes using RT-PCR and Western blotting. Equivalent measurements will be conducted *in vivo* using distinct approaches – volumetric measurements, bio-luminescent imaging of tagged engrafted lines and whole-body weight measurements over time. In addition, we will perform multi-timepoint tumour harvesting and downstream processing for transcriptomic and protein measurements.

This joint DPhil project will bring together Professor Stevens' expertise in drug delivery and advanced therapeutics with Professor Mills' proficiency in cancer biology. This synergy is designed to enhance the development of innovative treatment strategies for prostate cancer. Additionally, we plan on engaging clinical advisors who specialise in drug-resistant prostate cancer, who will provide critical insights and bridge the gap between research and clinical application.

Translational potential of the project.
This project spans bioengineering, cancer biology, and clinical oncology to innovate prostate cancer treatments and streamline their clinical translation. By developing locally injectable, biodegradable microparticles for sustained delivery of AR antagonists, it addresses a critical need in prostate cancer therapy. Sustained release of AR antagonists can help overcome drug resistance and enhance treatment effectiveness. Additionally, localized delivery minimizes systemic side effects, improving tolerability, patient compliance, and quality of life. Since both AR antagonists and polymer matrices proposed for the project are either clinically approved or GRAS, translation into practical medical applications would be significantly expedited.

Training opportunities
1. **Polymer synthesis.** Depending on the candidate's interest and expertise, they will have the opportunity to receive specialised training in polymer chemistry for the synthesis and fine-tuning of the polymer matrix. They will be guided and supported as necessary by experienced chemists within the Stevens group.
2. **Microparticles fabrication and characterisation.** The candidate will learn how to use microfluidic-assisted techniques to fabricate drug-loaded microparticles. Additionally, they will acquire skills to characterize these microparticles through various techniques, including but not limited to dynamic light scattering (DLS), scanning electron microscopy (SEM), degradation and release studies (biochemical assays, single-particle Raman spectroscopy).
3. **In vitro prostate cancer models.** The candidate will gain expertise in handling and characterising different preclinical prostate cancer models, from cell lines culture to patient-derived organoid preparation. The candidate will gain valuable expertise in advanced techniques in molecular biology and genomics, including but not limited to chromatin immunoprecipitation and sequencing (ChIP-seq), RNA sequencing (RNA-seq), and transcriptomics.
4. **In vivo prostate cancer models.** The candidate will undergo Personal License training to undertake cell-line engraftment (sub-cutaneously and orthotopically), and monitor changes in tumour volume, whole-body weight and murine health over time and in in vivo imaging.

The proposed DPhil project ensures an enriching academic experience within a highly multidisciplinary, collaborative, and translational environment. The student will not only acquire technical proficiency in both drug delivery and cancer biology, but also hone transferable skills typical of a doctorate programme, such as ethical research, data management, analysis and interpretation, science communication and many more. By working across disciplines and integrating diverse perspectives, the student will be primed to address complex issues effectively, making substantial contributions to both academia and real-world applications.

Ideal student background: While backgrounds in biochemistry or pharmaceutical sciences are preferred, this project welcomes individuals from all disciplines who possess a strong passion and inclination for multidisciplinary and collaborative research.

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17. Developing single-cell transcriptomics tools for PARP inhibitor resistance in BRCA1/2-deficient cells and tumours – Madalena Tarsounas

Primary Supervisor: Madalena Tarsounas
Additional Supervisors: Christiana Kartsonaki
Eligibility: Track 2 applicants are eligible to apply for this project

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

a. Characterize the single-cell transcriptomic landscape of olaparib-resistant *BRCA1/2*-deficient cells in culture.
The scRNAseq technology will enable us to generate gene expression profiles of single cells and to identify cell sub-populations with specific transcriptional signatures. To achieve this first objective, scRNAseq will be carried out in populations of olaparib-sensitive and -resistant *BRCA1/2*-deficient cells, already generated in Tarsounas lab. We will prepare libraries from each cell line, before and after olaparib resistance onset, to sequence between 7,000 and 10,000 cells using the standard protocol of the Chromium Single-Cell 3' gene expression profiling solution (10x Genomics).
Unsupervised clustering approaches will be developed to classify cells into sub-groups with specific signatures (e.g. immune response, metastasis etc.) and to monitor cell dynamics using algorithms for pseudotime analysis. We will apply this combination of analytical approaches to the cell lines that are sensitive or become resistant to Olaparib, and anticipate that this will allow us to identify cell clusters with unique patterns of gene expression, which could 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 model's 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.

Translational potential of the project
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.

Ideal student background: This project would be suitable for a student with a strong interest in bioinformatics, statistics and cancer biology. Experience in using statistical software such as R, other programming languages, or command line tools would be highly desirable. Familiarity with statistical concepts, computational biology resources, handling complex datasets and wet lab experience is desirable. Ideally the candidate would have some formal training in both biology or a related discipline and statistics or a related numerate discipline. A strong interest in developing strong programming and bioinformatics skills as well as knowledge of cancer genomics and biology is essential.

18. The role and mechanism of highly variable genetic factors in cancer risk and prevention – Ian Tomlinson

Primary Supervisor: Ian Tomlinson
Additional Supervisors: Christiana Kartsonaki
Eligibility: Track 2 applicants are eligible to apply for this project

Abstract
Identifying and characterising cancer risk factors is important for identifying individuals at increased risk of disease and working out how those risk factors operate to increase the chance of disease. Ultimately, that knowledge feeds through into cancer prevention. Genetic risk factors are largely unavoidable, but they can be mitigated. Since genetic factors provide particularly strong evidence for the mechanisms underlying carcinogenesis, they have great potential for developing new prevention measures, such as chemoprevention that could be used in the same way as cholersterol-reducing and blood pressure-lowering agents are widely used to reduce the risk of cardiovascular disease. Whilst studies over the last 10 years have made large inroads into identifying inherited cancer risk factors, much remains unknown, in part because the techniques used have not been able to analyse some types of genetic variation on a large scale. Nevertheless, we can now begin to study those variants, such as short or complex repeat sequences, owing to the growing number of cancer patients whose whole genomes have been sequenced. This project will search for inherited variation in repeated DNA sequences that increases the risk of colorectal and other cancers. The same sequences may also undergo acquired changes as tumours develop and help to drive cancer growth. The project will include computational analysis of large human data sets with genetic data (e.g. 100,000 Genomes, UK Biobank) and laboratory analyses. The balance between these types of work is flexible.

Ultimately, the project should identify mechanisms of tumour growth and strategies to counter those mechanisms. The project is novel, in that very little work has been performed in the specific area to date, but builds on multiple related studies performed by the supervisors that have identified >200 cancer-associated genes or polymorphisms to date.

Research objectives and proposed outcomes

Background

Many types of repeat sequence are present in the human genome and in some cases, that variation can affect the expression of genes involved in increasing cancer risk. The simplest repeats are arguably short tandem repeats (STRs, microsatellites) that comprise strings of repeated DNA bases. The length of the repeat varies considerably and studies have shown that this can affect gene expression and hence influence the risk of disease. However, we have not been able to study these sequences at scale in the past, because whole genome sequencing (WGS) data are needed for several thousand patients and controls. We can now start to address the issue of microsatellites and cancer risk. In short, we can perform a hypothesis-free search for associations between microsatellite allele lengths and gene expression in colorectal tissues (normal and tumour), and then measure the lengths of such microsatellites in large sets of cancer cases and population controls. Significant associations will identify cancer risk polymorphisms and can lead to additional functional studies in human cancers prone to acquire somatic microsatellite mutations and of the genes targeted by the change in gene expression. Strategies to use the data to prevent cancer will be developed for the longer term.

Project

1. Use existing information to identify locations of variable microsatellites in human genomes.
2. Develop simple methods to identify the lengths of the two alleles from short- and long-read sequencing data of constitutional DNA.

3. Use public and in-house reference data to link variation in microsatellites to expression of nearby genes in normal tissue and tumours
4. Identify a set of microsatellites associated with differential gene expression or function (e.g. polymorphic microsatellites or short repeats can occur in coding sequences or influence gene expression by affecting transcription factor binding)
5. Test those microsatellites for inherited differences between large sets of colorectal cases and controls, thus identifying cancer risk factors.
6. Extend the search to other types of repeat sequence or perform laboratory studies of selected target genes, depending on data obtained and preferences of the DPhil student.
7. If time permits, expand the analysis to include the role of microsatellite mutations acquired by caners, especially those with defective DNA mismatch repair that have an increased rate of microsatellite mutation.

Translational potential

The project will improve understanding of how this type of genetic variation contributes to cancer risk and to identify somatic driver mutations caused by STRs for different cancer types. In the long term these may contribute to predicting risk of cancer and in the development of treatment and prevention strategies.

Training opportunities

The student will receive full training in statistical and bioinformatics methods, in genetic epidemiology and genomics, as well as software commonly used in such analysis, such as R, Unix and command-line tools. Access to large genomic data sets will also require training in the use of secure research environments, e.g. UK Biobank, Hartwig Foundation, Genomics England. Training will also be provided, if desired, in relevant laboratory methods.

Ideal student background: None specifically required, but some computational background, interest or ability would be helpful.

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Return to Projects list

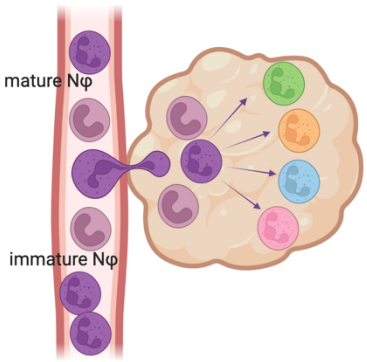
19. Spatiotemporal heterogeneity of neutrophil subsets in ovarian cancer – Irina Udalova

Primary Supervisor: Irina Udalova
Additional Supervisors: Sarah Spear
Eligibility: Track 2 applicants are eligible to apply for this project

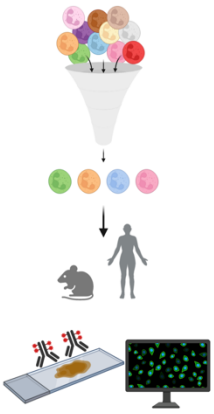
Abstract
Ovarian cancer is the 6th most common cancer in women, of which over 90% cases are epithelial high-grade serous carcinoma (HGSC) (CRUK). The majority of women are diagnosed with HGSC at stage 3-4 where the 5-year survival remains poor at 15-25%. HGSC disseminates throughout the peritoneal cavity generating secondary tumours, including on the omentum. Patients rely on surgery and chemotherapy to achieve remission, however even with PARP-inhibitor maintenance, acquired resistance is common and relapse rates are high. Therefore, approaches that circumvent therapy resistance are urgently needed. Neutrophils are the most abundant immune cell circulating in the human body and are actively recruited in ovarian cancer (1,2). A high neutrophil to lymphocyte ratio is a predictor of poor prognosis in ovarian cancer patients (3). As immune checkpoint blockade, targeted to lymphocytes, has shown no efficacy at clinical trial (4,5), it is critically important to understand how neutrophils might contribute to the progression of ovarian cancer. Neutrophils are typically seen as transcriptional inactive cells, with a short life-span that are rapidly recruited to inflammatory sites. However, recent work by us and others in the context of inflammatory disease have revealed neutrophils are heterogenous dynamic cells, transcriptionally imprinted by their microenvironment (6,7). Mounting evidence shows that the HGSC tumour microenvironment (TME) also modulates neutrophil function. Ovarian cancer-derived neutrophils have an extended life-span and immunosuppressive phenotype (2). They can produce neutrophil extracellular traps (NETs) and enhance tumour cell attachment (1). On the contrary, they can also upregulate costimulatory molecules and stimulate T cell IFNγ production (8). Pan-targeting neutrophils both improves and worsens survival, demonstrating neutrophil functional heterogeneity exists. To date, no *in vivo* transcriptional and functional characterisation of neutrophil subsets in ovarian cancer tumours has been performed.

Research objectives and proposed outcomes

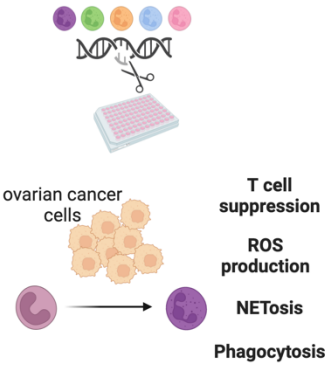
WP1: Neutrophil subsets transcriptionally programmed by ovarian cancer tumour



WP2: Validation and spatial characterisation of neutrophil subsets



WP3: Investigation of neutrophil subset function in tumour microenvironment



We hypothesise that neutrophils are transcriptionally reprogrammed by the HGSC TME leading to the development of distinct neutrophil subsets. Understanding the function of these subsets in the TME will uncover new avenues for targeting neutrophils.

Aims:

1. Characterise the neutrophil subsets within the HGSC TME and identify molecular pathways.
2. Unravel the spatial organisation of these neutrophil subsets in murine and human tumours.
3. Examine the role of neutrophil-specific molecular pathways in driving neutrophil functions within the TME.

Outcomes: This project will generate a blueprint of neutrophil molecular heterogeneity in ovarian cancer and uncover transcriptional networks that control neutrophil functions. We will develop novel neutrophil knockouts to validate the function of these subsets in disease progression. Targeting these subtypes remains completely unexplored as a therapeutic opportunity. These methods can uncover new avenues for targeting neutrophils that can be replicated in other cancers. (ii) Collaborations: This project underpins a newly set up collaboration, between Oxford and Imperial College. It will combine the unique expertise of Prof Udalova (Oxford) in the transcriptional networks that control the heterogeneity and function of neutrophils with an expertise in models of ovarian HGSC of world-leading ovarian cancer researcher, Prof McNeish. Dr Sarah Spear (RcoI) is a postdoctoral researcher specialised in characterising myeloid cells in both inflammation and TMEs. Her research interests and pilot data have laid a foundation for this proposal, bringing together Udalova team and McNeish expertise.

Translational potential of the project

Immune checkpoint blockade, targeted to lymphocytes, has shown low efficacy in ovarian cancer at clinical trials. T cell immunity, which is beneficial in tumours, is undermined by immunosuppressive myeloid cells. Thus, it is critically important to understand how these cells, and specifically less studied neutrophils, might contribute to the progression of ovarian cancer. This will help shaping specific therapies targeting neutrophil subsets. We have already identified a number of regulators that play a critical role in mediating neutrophil recruitment and their differentiation, as well as in effector functions, in inflammatory settings. More will be identified during the course of this project, specific to the ovarian cancer development. The inhibitors of these regulators or their activation pathways may prove beneficial for inflammation-induced cancer.

Training Opportunities: The student will be trained in the models of ovarian cancer as well as cutting-edge microscopy and spatial transcriptomics approaches to define the localisation of myeloid cell subsets within the tumour microenvironment.

Ideal student background;The applying student would be eager to learn new techniques and models as well as able to work independently and in collaboration. They ideally would have an immunology or genomics background and developed interest in cancer, innate immunity and mucosal immunology. A vibrant collaborative group is awaiting them and there is an opportunity to learn various cutting-edge techniques as well as basing new discoveries on well-established models in the group.

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20.BLOod Test Trend for cancEr Detection (BLOTTED): an observational and prediction model development study using English primary care electronic health records data – Pradeep Virdee

Primary Supervisor: Pradeep Virdee
Additional Supervisors: Brian Nicholson and Eva Morris
Eligibility: Track 2 applicants are eligible to apply for this project

Abstract
Blood tests are commonly requested in NHS General Practice. Blood tests might be ordered when the patient attends their GP with symptoms or signs, to monitor a known medical condition, or as part of a "health check". Some clinical guidelines for GPs include recommendations that they should investigate for cancer if a blood test is lower or higher than a normal level. These recommendations are only helpful for a small number of cancers, such as bowel or pancreatic. Over time, a patient can build up a sequence, or trend, of blood test results. This trend might tell GPs more information than single blood test results. For example, a small drop from a steady trend could be more useful than waiting for the blood test to drop below a fixed level. However, the research has not yet been done to tell us which approach is most helpful to find patients who need referral to hospital for cancer investigation. In this research, we will explore if blood tests trend can detect patients with cancer better than single blood tests and check which patient groups trend is more helpful in and for what cancers, with primary focus on digestive and blood cancers.

Research objectives and proposed outcomes
Background: A recent clinical review confirms that simple blood tests have an important role in identifying patients for cancer investigation [1]. However, analysis of National Cancer Diagnosis Audit in Primary Care data suggests that primary care investigations may delay referral [2]. Smarter use of blood tests to select patients for further cancer investigation could increase cancer yield and reduce unnecessary referrals. Our recent research highlighted that trends over time in serial blood tests could be more useful than single blood tests and non-specific symptoms to select patients for colorectal cancer investigation, with our our colorectal cancer prediction models having good predictive ability [3,4]. However, trends are subtle so difficult to spot and may exist for various cancers.
Aim: To utilise trends in blood tests from primary care for early detection of cancer.

Objectives: There are three main objectives:
1) *identify trends among repeated blood tests indicative of cancer* – the student will learn of smoothing techniques, such as LOWESS, to graphically describe trends in each blood test, both overall and by personal, clinical, and cancer characteristics (e.g. age, sex, comorbidity, diagnosis route, site, stage). Collaborators: the Big Data Institute will collaborate on data curation and understanding of electronic health records data.
2) *assess predictive ability of blood test trends for different cancer types* – the student will learn of dynamic models, which utilise repeated measures data for assessing clinical outcomes. These include statistical models, such as joint modelling. Collaborators: the Big Data Institute will collaborate on the interpretation of repeated measures data from national datasets.
3) *develop and test prediction models utilising blood test trend to optimise patient selection for referral* – the student will learn of the intricacies of developing and testing dynamic prediction models and their clinical application. Collaborators: the Big Data Institute will collaborate on the interpretation of results from national datasets.

Data: Data from ~28 million patients from the CPRD primary care database is available to develop the models. It includes information on patient characteristics, deprivation, blood tests, symptoms, medications, cancer

diagnosis, and other variables over 2000-2019. It is linked to the National Cancer Registration and Analysis Service, Hospital Episode Statistics databases, and Office of National Registration death database.
Outcomes: The main outcome will be prediction models that incorporate blood test trend for cancer risk. Outputs will include peer-reviewed journal publications for each objective separately and conference presentations.
Academic value: This research will develop an evidence base for blood test trend for cancer detection and inform clinical practice. The DPhil candidate will develop leadership and research skills in various areas, including primary care, electronic health records data, patient and public involvement, and more. The student will grow their academic publication record and research networks at courses and events. Collaborations in this research will provide direct access to further multidisciplinary teams to improve efficiency in conducting this research.

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21. Manipulating intratumoural dendritic cell fate to enhance anti-cancer immunity – David Withers

Primary Supervisor: David Withers
Additional Supervisors: Audrey Gerard
Eligibility: Track 2 applicants are eligible to apply for this project

Abstract
Targeting the molecular pathways that restrain T cells has achieved striking results in the treatment of some cancers, particularly those where the tumours contain numerous mutations that can be recognised by the immune system[1-3]. However, despite improving control of these more immunogenic tumours, the majority of patients still fail to durably respond[4]. Furthermore, there are many common cancers for which immunotherapy has had minimal impact, even in the short term[5]. Here it is likely that the anti-tumour T cell response failed to either properly establish, or reach a threshold at which immune checkpoint blockade could enhance the response. This may reflect a limited number of mutations visible to T cells, dominant suppressive mechanisms or a combination of both these factors. **Understanding why the T cell response is impeded and identifying approaches to overcome this failure to generate robust anti-tumour immunity, remains vital to supporting the design of better treatments for cancer patients.**

Tumours excel at subverting the anti-tumour response through multiple mechanisms[6, 7], for example creating tissue niches that exclude T cells or inhibiting T cell functions to render these cells ineffectual. Furthermore, productive CD8 T cell responses are not generated in cellular isolation, rather, they require a supportive team of other immune cells, in which dendritic cells (DCs) are key[8]. Activated DCs carry tumour antigens to draining lymphoid tissue to prime and expand the effector T cells that traffic to the tumour and kill cancer cells[9]. Alongside this critical role in establishing the anti-tumour T cell response, it is now appreciated that interactions between responding T cells and DCs within the tumour itself, further regulate T cell effector functions[10, 11]. Thus activated DCs lie at the heart of anti-tumour response, controlling the magnitude and fitness of the response. **However, we recently discovered some activated DCs become trapped within tumours, rather than migrating to lymphoid tissues, resulting in the formation of a distinct intratumoural DC compartment that appears to be dysfunctional**[12]. Thus DCs join the expanding list of immune cells that can become corrupted by the tumour microenvironment. Determining how best to block the formation, retention or functions of these defective DCs presents an exciting new approach to enhancing anti-tumour immunity.

We hypothesise that cancers: a) exploit mechanisms that cause the retention of activated DCs within tumours, thus impeding T cell priming in draining lymphoid tissue and b) accumulate altered DCs that further impede intratumoural effector T cell function.

The overarching aim of this project is to understand the mechanisms controlling DC fate within the tumour and how this impacts support for intratumoural T cells**.** Deciphering how and why this occurs has the potential to support the design of immunotherapy combinations tailored to ensure robust T cell responses. This is of particular relevance to cancers characterised by a limited T cell infiltrate, which includes the majority of colorectal cancers[13].

Firstly, we will interrogate the functions of the activated DCs that become retained with tumours, exploiting photo-labelling approaches developed in the Withers Lab to specifically capture cells based upon time spent within the tumour microenvironment[14, 15]. While this work is focused on colorectal cancer, including the use of orthotopic models implanting mouse tumour organoids, the relevance of dysfunctional intratumoural DCs will be assessed across other cancer types using other models established in the lab. Building from detailed transcriptomic resources, DCs from photo-labelled tumours will be characterised by spectral flow cytometry and

compared with DCs in draining lymphoid tissue, including those activated DCs that have demonstrably migrated (utilising photo-labelling to track cells). We will functionally test the ability of these DC populations to prime CD4 and CD8 T cells *ex vivo* and assess their provision of costimulatory and coinhibitory ligands. Secondly, using cutting-edge imaging approaches, alongside established models that support targeted manipulation of tumour cells and the local stroma, we will investigate the cellular niches in which DCs reside and seek to define the microenvironment(s) in which DCs become trapped.

Informed by the cellular interactions observed, alongside leveraging scRNA-seq resources, we will explore the mechanisms that orchestrate DC retention (e.g. blocking Abs *in vivo* combined with photo-labelling and tracking of cellular egress). We will then look to more definitively test the most promising candidates through genetic approaches. Finally, we will aim to identify immunotherapy combinations that boost DC support for the T cell response and, when combined immune checkpoint blockade, drive durable and systemic protection. Using the array of tumour models established within the lab, robust anti-tumour immunity will again be tested *in vivo*.

Research objectives and proposed outcomes

The specific research objectives for this Project are as follows:

1. Investigate DC fate over time, specifically in models of colorectal cancer, but further compared to other cancer types to establish broader relevance and tissue/site specific cues.
2. Define the functional capacity of different intratumoural DC populations and the mechanisms through which these are orchestrated.
3. Determine the cellular niches in which DCs reside and test the role of interactions with different stromal compartments in mediating DC retention.
4. Develop approaches to manipulate intratumoural DC fate and enhance the anti-tumour response.

The principle outcome of this project will be detailed mechanistic insight into how fate and function of DCs is controlled within the tumour microenvironment, fundamental knowledge that underpins the success of anti-tumour T cell responses. We will identify approaches to manipulate DC behaviour and test combinations of immunotherapies designed to enhance the anti-tumour response in pre-clinical models of colorectal cancer. Collectively, this research will inform of potential therapeutic approaches to enhance responses in cancer patients currently in desperate need of better treatments.

Translational potential of the project

Combinations of immunotherapy, tailored to the type and stage of cancer, offer clear potential in refining the clinical impact of immune checkpoint blockade and improving cancer patient treatment. Rationalising these immunotherapies requires detailed mechanistic understanding of the critical steps that drive and sustain the anti-tumour response. This is a fundamental research project that aims to help address this critical knowledge gap and support the development of better therapies for colorectal cancer patients.

Training opportunities

This project will provide a wealth of training opportunities and is ideal for students wishing to develop expertise in studying immune responses to cancer utilising the most advanced *in vivo* models available. Extensive training in an array of cutting-edge experimental approaches (e.g. photo-labelling, conditional targeting of molecular interactions, fate-mapping) across multiple cancer models (intradermal, orthotopic, mouse-tumour organoid) will be provided. Transcriptomic approaches (e.g. scRNA-seq), alongside flow cytometry (including spectral flow) and imaging approaches (multiplex imaging including Cell DIVE, MIBIscope) will be used to interrogate immune responses, alongside *ex vivo* functional analyses. Collectively this will ensure comprehensive training in the core approaches required to interrogate immune responses, with appropriate appreciation of immune cell phenotype, spatial distribution, cellular interactions and functional relevance.

This research project benefits from close interactions with multiple other labs, both locally (Gerard, Coles, Leedham) and nationally (Zelenay). This project further complements the research focus of a Wellcome

Discovery Award (led by DRW) and the student will be fully integrated into the regular meetings underpinning this team research programme, coordinated between the Withers Lab and the labs of Menna Clatworthy and Sophie Acton (Discovery Award CoApplicants).

The Withers Lab is highly collaborative and adopts a team science approach. There is extensive technical support for the *in vivo* research and the lab has a long track record in providing a supportive environment in which post-graduate students flourish.

Ideal student background: The student requires basic knowledge in immunology. An enthusiasm and interest in cancer immunology is essential. Knowledge/experience in tumour immunology is obviously advantageous. Experience in the use of immune assays, alongside in vivo or ex vivo experiments is necessary. The student should be willing to work with mouse models of cancer.

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22. Developing a vaccine for ovarian cancer prevention – Nancy Zaarour

Primary Supervisor: Nancy Zaarour
Additional Supervisors: Ahmed Ahmed
Eligibility: Track 2 applicants are eligible to apply for this project

Abstract
High-grade serous ovarian cancer (HGSC) is the most lethal histotype of ovarian cancer, largely attributed to late-stage presentation. with a lifelong risk, in some cases, exceeding 40%, prompting current clinical recommendations for prophylactic surgery by the age of 35, emphasizing a substantial need for the development of preventative strategies for ovarian cancer including vaccination. Accumulating evidence emerging over the past two decades strongly indicate that most HGSCs are derived from the fallopian tube. Moreover, frequent mutations in the *TP53* tumor suppressor gene, arise at the premalignant lesions called STICs in the fallopian tube (FT), the earliest step of HGSOC development, in nearly 100% of cases, leading to further oncogenic mutations. However, whether or not local immunity in the fallopian tube plays a role in modulating transformation or establishing serous ovarian cancer has remained unknown. Based on our recent findings, we have now discovered that tissue resident memory T cells (TRMs) that reside in non-cancerous fallopian tubes react to tumour organoids derived from omental metastases in the same patient. Moreover, these memory cells induce apoptosis in tumour-derived organoids indicating that they possess cytotoxic ability. In this project, we propose to use our established models of fallopian tube organoids, ovarian cancer organoids and epithelial-immune cell co-culture methods to test the hypothesis that a vaccine that we designed based on ovarian cancer tumour associated antigens (TAAs) and recurrent mutations could be effective in preventing early transformed cells in the FT . We believe that a vaccine targeting TRMs would be highly effective in constraining the local spread of STICs by maintaining an immune-equilibrium in the FT. We will undertake this essential pre-clinical work as a stepping-stone towards clinical testing of our proposed vaccine.

Research objectives and proposed outcomes
Aim 1): To evaluate the immunogenicity and memory recall of ovarian cancer specific peptides in patients/and Healthy individuals with potential to develop a specific T cell response against these peptides. To this end, T cells isolated from PBMCs, fallopian tubes and tumour infiltrating lymphocytes (TILs) will be interrogated for their ability to respond to our selected peptides.
Subaim1) using ex vivo assays, we aim to assess the immunogenic potential of our selected peptide pools on Naïve T cells from peripheral blood of healthy blood-donor females aged 30-40 (to mimic the target vaccine population).
Subaim2) To elicit a secondary recall response in patients with ovarian cancer: We will investigate whether these peptides can activate memory cells from non-cancerous fallopian tubes of ovarian cancer patients, exploring potential memory response for ovarian cancer antigens. Such memory could be acquired through prior exposure to premalignant lesions. We will also test TILs from the same patient for comparison.

Aim 2): Using our extensive experience in T-cell-organoid co-culture systems that we have recently developed in our lab, we will Test the hypothesis that either *in vitro* primed naïve T cells or induced memory cells are cytotoxic to autologous ovarian cancer organoids and early-transformed fallopian tube epithelial cells.

Proposed outcomes: (i) Testing the efficacy of cancer vaccines in preclinical models prior to clinical trials is essential for successful vaccine development. However, such testing is very difficult since animal models do not faithfully recapitulate human cancer-immune interactions. Previous work in our lab lead to the development of organoid models that faithfully represented non-cancerous fallopian tube epithelial (FTE) composition (1, 2), as

well as 3D cancer organoid systems that recapitulate the *in vivo* genomic and transcriptomic features of ovarian cancer (3). Along with our expertise in T cell immunology, a tailored method has been recently developed in our lab to maintain the phenotype and to expand newly identified subsets of T cells termed TRMs homing the FTE. We propose to use our established models of fallopian tube organoids, ovarian cancer organoids and epithelial-immune cell co-culture methods to allow for testing of peptides-primed T cells' cytotoxicity against patient-derived ovarian cancer organoids, allowing for the selection of peptides/epitopes for vaccine development. Therefore, facilitating a successful completion of the proposed work that has several promising proposed outcomes. First, at preventing the onset of ovarian cancer development, a significant decrease in the number of new cancer cases by targeting high-risk populations will be expected. Prevention of ovarian cancer development in a clinical trial that should be undertaken after completion of the preclinical work, by envisioning a vaccination of patients prior to surgery (reduction in size and prevalence of STICs in the vaccinated groups compared to non-vaccinated will be expected). Second, early elimination of precancerous cells, by priming/boosting the local and systemic immunity, TRMs in the FTE will recall a memory response, recognize, and destroy cancer cells harbouring these early mutations and preventing progression to fully established cancer. Third, providing a durable immune response, by establishment of a long-lasting immunity and generation of memory T-cells that remain vigilant and ready to attack precancerous cells that present these proposed TAAs and neoepitopes at very early-stage of ovarian cancer. Therefore, reducing mortality, need for surgery, chemotherapy, and radiation, leading to a better quality of life.

This studentship would greatly facilitate collaboration opportunities with clinicians, academics and vaccine experts for the students by providing financial support, opportunity to work closely with experienced scientists. It can provide invaluable guidance, enhance the quality of the research, and help students navigate complex academic challenges. Additionally, networking with other scholars in the field can lead to interdisciplinary research. In addition to the practical skills in immuno-oncology and vaccine development they can acquire, access to resources including tissue cultures, FACS facilities and data analysis, equipment for molecular and cell biology. It also offers the opportunity to develop other transferrable skills through access to courses offered by the Medical Sciences Division, the WIMM, and the wider University. It can allocate a more focused mentorship, research time and professional development. These benefits collectively contribute to producing high-quality, impactful research that advances knowledge in T cell immunology, sequencing technologies, bioinformatics, cancer biology and the most advanced preventative vaccine strategies.

Training opportunities

1. Clinical samples processing and isolation of primary immune cells from matched FTs, tumours, and blood samples.
2. Developing immunogenicity assays for TAAs and mutated peptides screening:
3. in vitro assays include T cell expansion, flow cytometry, cell sorting and ELISpot cytokine release assays.
4. In vitro Dendritic cells/T-cell coculture assays.
5. Generation of FT and tumour 3D organoids.
6. Cytotoxicity assays using T-cell organoids coculture methods, followed by live imaging (Incucyte).
7. CRISPR-Cas9 knockout and single nucleotide editing.
8. Single-cell sequencing

References

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3) Yang X, Artibani M, Jin Y, Aggarwal A, Zhang Y, Muñoz-Galvan S, et al. A 3D microtumour system that faithfully represents ovarian cancer minimal residual disease. bioRxiv. 2023:2023.07.15.549155.

Return to Projects list

DPhil in Cancer Science – Clinical/ Medical Undergraduate Project Booklet

Introduction

This handbook provides an overview for prospective students looking to study for a DPhil in Cancer Science starting in 2025 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

The DPhil in Cancer Science has four different tracks with two different pathways of entry. This booklet will focus on Tracks 1 and 2; clinical trainees and medical undergraduates who will **undertake a 3-year research project selected from this advertised 'DPhil in cancer science – Clinical/ Medical Undergraduate booklet'.**

Post-graduate medical trainees and undergraduate medical students are eligible to apply for the fully funded studentships at the home rate. All students are admitted directly to work under the supervision of a Principal Investigator who is formally appointed as the DPhil supervisor.

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).

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 £21,000 per annum.

International applicants are eligible; however, funding is limited to the home level for this programme and therefore international applicants would need to source further funding.

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 Monday 2nd December 2024.** Shortlisted students will be invited to interview in January. It is strongly suggested that students contact supervisors of projects they are interested in applying for prior to application.

Projects

Projects are listed below. Clicking on a project title below will take you to the relevant project page.

Contents

1. ADP-ribosyl hydrolase as a biomarker for PARP inhibitor sensitivity/resistance – Ivan Ahel

Primary Supervisor: Ivan Ahel
Additional Supervisors: Ahmed Ahmed
Eligibility: Track 1 and 2 applicants are eligible to apply for this project

Abstract of the project

To protect the genome from damage organisms have evolved a cellular defence mechanism termed the DNA damage response (DDR). The DDR includes a diverse set of signal transduction pathways and effector proteins that act to sense DNA lesions and effectively repair the damage, limiting the propagation of genomic instability. Exploiting DDR pathways to specifically target and kill cancer cells has become an attractive therapeutic avenue within cancer research. This is exemplified by the synthetic lethal interaction between PARP inhibition and *BRCA1* or *BRCA2*-deficient tumours[1]. Ivan Ahel (co-supervisor on this project) laboratory recently identified HPF1 protein as a novel interactor and critical regulator of PARP1 ADP-ribosylation activity upon DNA damage[2]. Functionally, HPF1 suppresses DNA damage-induced hyper auto-modification of PARP1 and promotes *in trans* ADP-ribosylation of histones and many other proteins involved in regulation of genome stability. They further demonstrated that HPF1 is a critical specificity factor that allows modification of target proteins by PARP1 on serine residues (Ser-ADPr)[3,4]. Crucially, the work also identified ARH3 as a hydrolase which specifically removes Ser-ADPr[5] and further showed that Ser-ADPr is the major form of ADP-ribosylation following DNA damage[6]. Taken together, the insights surrounding Ser-ADPr open a large, exciting, and novel area of research into the fundamental understanding of the pathways regulated by this modification. Strikingly, our recent data show that ARH3 knockout in model cell lines associates with PARP inhibitor (PARPi) resistance, while ARH3 overexpression is associated with PARPi sensitivity[7]. Based on these results, we hypothesize that ARH3 activity and protein levels affect sensitivity to PARPi, thus representing; i) a predictor for the success of these therapies and, ii) a novel target for further drug development. Currently, PARP inhibitors are used to treat ovarian cancer and several other cancers, and we therefore propose to test the hypothesis that ARH3 expression might be a useful diagnostic tool with which to stratify cancer patients into sub-groups that will be sensitive/resistant to PARPi treatment with a particular focus on ovarian cancer. The mechanism of sensitivity/resistance of cells with deregulated ARH3 expression cells to PARPi is unknown, and elucidating this mechanism will be another goal of this proposed work.

Research objectives and proposed outcomes

Objective 1. Characterise the effect of ARH3 under- and overexpression in a series of model and primary cancer cell lines on PARP inhibitor sensitivity/resistance. We will collect and test a variety of ovarian cancer cell lines, profiling them for ARH3 protein expression levels and then treating with several different PARPi of varying PARP-trapping capabilities (olaparib, talazoparib, veliparib). To determine the impact of ARH3 protein levels on PARPi vulnerability, we will not only assess drug sensitivity and levels of PARP1, PARG, and ARH3 across a panel of ovarian cancer cell lines, but also assess the impact of systemically varying ARH3 by knockdown, knock out and inducible overexpression in HGSOC lines of defined genotype, including Ovcar8 (BRCA1/2 wt, PARPi resistant), PE01 (BRCA2-mutant, PARPi sensitive), Kuramochi (BRCA2-mutant, PARPi partially sensitive) and COV362 (BRCA1-mutant, PARPi sensitive). Rescue experiments with wild type vs. catalytically inactive ARH3 will assess the suitability of ARH3 as a target for the development of inhibitors.

Objective 2. To determine the frequency of ARH3 gene alterations in a larger set of HGSOC samples, we will: i) interrogate data of an ongoing whole exome sequencing study of 504 ovarian cancers searching for ARH3 and PARG copy number alterations and mutations; and ii) perform semi-quantitative detection of ARH3, as well as of PARG, PARP1 and PAR, by immunohistochemistry (IHC) on two independent sets of tissue microarrays (TMAs) containing a total of 1200 ovarian cancers. To augment these analyses, which will be limited by the small number

of tumors treated with PARPi, we will also evaluate levels of ARH3, PARG, PARP1 and PAR in patient-derived xenograft (PDX) models that have been assayed for response to single-agent PARPi, including ones that have a high HRD score but did not respond. This objective will be performed in co-supervisor (Prof Ahmed Ahmed) laboratory at the Nuffield Department of Women's & Reproductive Health, University of Oxford.

Objective 3. Elucidating the mechanistic basis for the sensitivity/resistance of cells with deregulated ARH3 expression cells to PARPi (modulation of the PARP-trapping, regulation of DNA repair pathway choice, regulation of the chromatin structure/epigenetic marks). For these studies we will use largely cell biology/biochemical and genomics approaches.

Translational potential of the project

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

Training opportunities

The student will have opportunities to train in diverse set of methods including cell biology/cell culture approaches for structure/function analyses, well-established cell survival assays that we be applicable for wide range of cell toxicity studies, immunohistochemistry methods and patient-derived xenograft (PDX) models.

Ideal student background: Knowledge of some aspects of cancer biology and basic molecular biology techniques. Interest in molecular mechanisms underlying cancer.

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Return to Projects list

2. A Functional Genomics Approach to Decipher GSC-Macrophage Interactions for Enhanced GBM Therapy – Sneha Anand

Primary Supervisor: Sneha Anand
Additional Supervisors: Daniel Ebner
Eligibility: Track 1 and 2 applicants are eligible to apply for this project

Abstract
Glioblastoma (GBM) represents a formidable challenge in oncology, characterized by rapid growth and an immunosuppressive tumor microenvironment (TME). Tumor-associated macrophages and microglia (TAMs) are pivotal components within the GBM TME, driving immunosuppression and tumor progression. Despite promising advances in immunotherapies across various cancers, their efficacy in GBM is hampered by this immunosuppressive milieu. This study aims to elucidate the intricate interplay between patient derived glioma stem cells (GSCs) and TAMs. Through a comprehensive genome-wide CRISPR screen, we seek to identify tumor-intrinsic genes crucial for macrophage-mediated tumor eradication via phagocytosis.

Introduction
Glioblastoma multiforme (GBM) is the most aggressive primary brain tumor, marked by rapid growth and a highly immunosuppressive TME. Standard treatments, including surgical resection followed by chemoradiotherapy, result in a median overall survival of only 14 months. While immune checkpoint inhibitors (ICIs) have improved outcomes in various cancers, they have shown limited efficacy in GBM due to the highly immunosuppressive immune TME composed primarily of microglia and macrophages, collectively known as TAMs. Recent research has identified TAMs as effector cells for tumor cell phagocytosis in response to CD47 blockade, but variability in this response suggests additional unknown signals. Through this study we aim to understand the interaction between glioma stem cells (GSCs) and TAMs using a comprehensive genome-wide CRISPR-Cas9 screen to identify tumor-intrinsic genes essential for macrophage-mediated tumor killing through phagocytosis.

Background
Immunotherapies, including immune checkpoint inhibitors (ICIs), have limited efficacy in glioblastoma (GBM) due to tumor-associated macrophage (TAM)-mediated immunosuppression (Mantovani, A et al, 2017). Macrophages play a dual role, either promoting tumor growth or facilitating its destruction, depending on external signals. A key mechanism is the “don’t eat me” signal, primarily involving the CD47 protein on cancer cells. CD47 binds to signal regulatory protein alpha (SIRPα) on macrophages, inhibiting phagocytosis and enabling cancer cells to evade immune detection (Chao et al. 2012). This signalling is typically present in normal cells but is overexpressed in tumor cells, helping them avoid immune destruction.

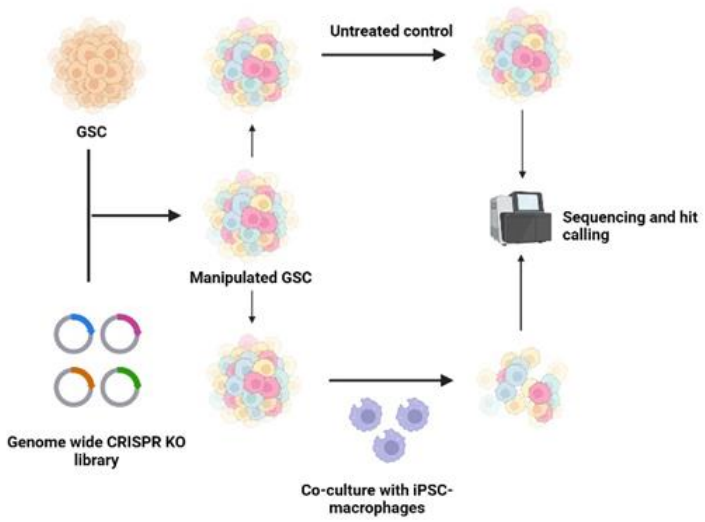
Drugs targeting CD47 can enhance macrophage phagocytosis by blocking the CD47-SIRPα interaction, thereby promoting tumor clearance. Similarly, another “don’t eat me” signal, CD24, interacts with Siglec-10 on macrophages, transmitting inhibitory signals that reduce macrophage phagocytosis (Barkal, A et al 2019). Blocking CD24-Siglec-10 interactions has shown potential in enhancing macrophage-mediated killing of tumor cells in other cancer types.

Despite these insights, the precise dynamics governing GSC-macrophage interactions and the essential tumor-intrinsic features facilitating macrophage-mediated tumor elimination remain obscure. This study proposes a comprehensive genome-wide CRISPR screen to unveil genes within GSCs pivotal for macrophage-mediated killing. Utilizing patient-derived GSCs and iPSC-derived macrophages, we will conduct co-culture experiments coupled with CRISPR-mediated genetic screens to delineate the genetic landscape essential for macrophage-mediated tumor cell clearance.

Methodology

1. **Genetic Manipulation of GSCs and Co-Culture screen with iPSC-Derived Macrophages/Microglia**: Patient-derived GSCs will be transduced with a whole-genome CRISPR-Cas9 knockout library.

   Manipulated GSCs will be co-cultured with iPSC-derived macrophages/microglia to investigate key genetic interactions essential for macrophage-mediated phagocytosis.



2. **Functional Validation**: Identified candidate genes will undergo functional validation through knockout studies to confirm their roles in macrophage-mediated killing of GSCs and their potential as therapeutic targets.

Fig 1: Methodology for CRISPR screening

Expected Outcomes and Significance

This study anticipates uncovering novel tumor-intrinsic genes crucial for macrophage-mediated tumor eradication in glioblastoma (GBM). Functional validation of these genes will provide insights into their roles in GBM progression and their potential as therapeutic targets. By disrupting the immunosuppressive crosstalk between GBM cells and tumor-associated macrophages (TAMs), particularly targeting the "don't eat me" signals like CD47 and CD24, this research holds promise for enhancing the efficacy of existing therapies and offering new avenues for GBM treatment. While monoclonal antibody therapies utilizing antibody-dependent cellular phagocytosis (ADCP) show promise in immunotherapy, challenges persist in GBM due to the low phagocytic activity of macrophages within the tumor microenvironment (TME) and the expression of anti-phagocytic factors by GBM cells. By employing a functional genomic approach to elucidate tumor-macrophage interactions in GBM, this study seeks to identify key tumor proteins involved in these interactions, including both "don't eat me" and "eat me" signals, thereby paving the way for targeted therapies. This research aims to enhance the understanding of macrophage-tumor interactions in GBM and identify potential therapeutic targets, offering transformative implications for GBM treatment.

Training Opportunities

This research project offers valuable training opportunities in CRISPR-based cell screening, cell biology, molecular biology, and bioinformatics analysis. The student will learn essential techniques for gene editing, high-throughput screening, and cellular manipulation. They will gain hands-on experience in cell culture, molecular biology techniques, and an introduction to bioinformatics data analysis. The project will enhance their skills in experimental design, data interpretation, literature review, and scientific writing. Overall, this project provides a solid foundation for their DPhil studies and future research in cancer biology and therapeutics.

Ideal student background: A student with a background in biomedical sciences, cancer biology, or a related field is suitable for this project. The project requires some basic understanding of molecular and cell biology. While it is not expected for the student to have expertise in all areas, a combination of theoretical knowledge and practical experience in the mentioned fields will enable them to contribute effectively to the project's delivery. Additionally, the student should possess critical thinking skills, attention to detail, and the ability to work independently as well as part of a research team. Strong communication and organizational skills are necessary for effective collaboration and project management.

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3. Multiomic Data Integration and Visualisation for the UK Brain Matrix Glioma Project – Olaf Ansorge

Primary Supervisor: Olaf Ansorge
Additional Supervisors: Stephen Taylor
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract of the project
Gliomas are rare cancers of the brain that are currently incurable [1]. They are a CRUK and national research priority. A multicentre approach is essential for trial design and evaluation of novel diagnostic technologies. To achieve this, the Tessa Jowell Brain Matrix (TJBM) has been established, representing ten centres of excellence across the UK [2]. The molecular neuropathology arm is led by Oxford in partnership with Genomics England and forms the framework for this DPhil project. The project is ideal for a candidate with previous experience or aptitude in bioinformatics. The purpose of the research is to devise bioinformatics solutions to comparative biological data analysis and visualisation, using a dataset of n=500 cases of glioma comprising paired tumour/blood short-read (Illumina) and long-read (Nanopore) sequencing, epigenomic methylation chip (Infinium), digital microscopy, and, in a subset of cases, cell-free circulating DNA, mass spectrometry and magnetic resonance imaging (MRI) data. This is the world's first systematic study of such a comprehensive dataset and analysis will be done in partnership with Genomic England's "Cancer 2.0" project team. The student will have the opportunity to make a substantial contribution to our understanding of how glioma genotypes relate to tissue phenotypes and how "near-patient" rapid Nanopore sequencing technology could be implemented for optimal and "real-time" glioma precision diagnostics and disease monitoring [3]. A challenge in this area of research concerns data analysis and visualisation; here, the student will have the opportunity to learn cutting-edge bioinformatic approaches, including machine learning and augmented reality-assisted visualisation of relational datasets spanning scales and modalities [4-6]. The latter will offer collaborations with industry.

Research objectives and proposed outcomes
(i) Academic Value: One of the major challenges for the realisation of "precision oncology" in glioma management concerns the identification of individually prognostic and predictive biological datasets from large-scale, so-called multiomic datasets (the term multiomics is used to describe the acquisition of multiple unbiased datasets such as genomics, metabolomics, proteomics from an individual's disease state or from a cohort of people with similar diseases). There are three challenges: (a) to identify what is relevant, (b) to be able to visualise (that is, understand) how data relate to each other, and (c) how to make this information accessible on an 'as-needed' basis in the clinical or research setting. The objective of this project is to use the multidimensional Brain Matrix dataset from n=500 people with gliomas and overcome the described challenges of data integration, distillation and visualisation. Specific outputs will be (a) comparison of short-read and long-read sequencing data, (b) comparison of long-read epigenomic classification with legacy EPIC bead-chip classification, (c) development and application of a machine learning process that relates genomic data to digital microscopy data to explore which genomic signatures may be predictable from digital microscopy data.

(ii) Collaborative Value: This award will form new collaborations between academia, the NHS and industry. Specifically, the student will benefit from placements with Genomics England and industry (see below) and become a member of the "LR CASe Detectives" group of the Central & South Genomic Medicine Service Alliance. Further, the project will bring glioma multiomic science into Oxford-led bioinformatics platforms (Oxford Cancer Translational Data Platform and Multidimensional Viewer) and thus establish new

collaborations across the Oxford biomedical campus. The supervisory team will include bioinformaticians, geneticists, biochemists, clinicians, neuropathologists and radiologists.

Translational potential of the project
The translational potential is clear: to make the multiomic data acquired by the UK's flagship glioma project, Tessa Jowell Brain Matrix, accessible to researchers and clinicians and provide evidence for the systematic implementation of 'near-patient, real-time' NHS glioma diagnostics and monitoring in support of novel trials of 'personalised' disease-modifying therapies.

Training opportunities
The student will acquire knowledge in modern data sciences as applied to oncological biological datasets spanning diverse scales and modalities, as outlined by Swanson et al., Cell 2023 [4]. Whilst the project will focus on in silico methods and solutions, the student will have opportunities to learn principles of data generation from biological samples (that is, pathways from sample collection via quality control to primary data generation) as it is important for the generation of models and visualisation tools to understand 'real-world' biases of data acquisition and curation. The student will have the option of placements with Genomics England, BrainLab and London Geometry.

Ideal student background : Evidence of some bioinformatics or programming experience must be provided. The ideal candidate has an MSc in biological sciences and demonstrable experience of bioinformatics as applied to datasets derived from single-cell or bulk-tissue genomics or other 'omics' approaches, such as automated image analysis pipelines. Due to the collaborative nature of the project, the successful candidate must have excellent communication skills.

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4. Primary Care presentations, referrals and cancer detection: understanding drivers to equitable access – Claire Bankhead

Primary Supervisor: Claire Bankhead
Additional Supervisors: Brian Nicholson
Eligibility: Track 1 applicants are eligible to apply for this project

Abstract of the project
Primary care is the first point of contact for most people who experience symptoms and are diagnosed with cancer. Therefore, this research will be focused on health-seeking behaviours, investigations, referrals and outcomes for patients in the primary care setting.

Sociodemographic variation in factors that may influence cancer outcomes have been observed at numerous points along the diagnostic pathway. Understanding the size of these differences, and who they affect is an important step to reducing inequalities associated with health-seeking behaviours, investigations, referrals and outcomes, including survival. Data from primary care consultations in over 20 million people are available for epidemiological analysis.

Research to understand how behaviours, beliefs and experiences of patients and clinicians can impact on the diagnostic journey is needed to minimise disparities in cancer diagnosis. Using qualitative or mixed methods approaches, this project has the scope to develop a deep understanding of the features or mechanisms that drive inequalities in cancer diagnosis.

Opportunities to modify behaviours or processes may be identified by this research, and hence lead to the development of relevant interventions or policies to improve the diagnostic experience for patients with symptoms associated with cancer.

This research could be designed with a broad focus across multiple cancer types, or could focus on one or a few cancer sites, or groups within the population.

Eliminating age, sex, and deprivation inequality in cancer stage at diagnosis could make a substantial contribution to achieving the national target to diagnose three in four cancers at an early stage by 2028.

Research objectives and proposed outcomes.
There is scope for the successful candidate to develop research objectives within the broad framework of CRUK Oxford Cancer Centre's Early Detection theme. The Early Detection theme includes a range of projects which include harnessing routinely collected data to undertake bespoke projects including focusing on symptom pathways, management in primary care and cancer outcomes. The successful candidate will be supported to develop and lead research focusing on symptom presentations, testing in primary care, referrals to specialist care and cancer outcomes.

Project themes may include:

- Descriptive and explorative analyses to describe relationships between symptom presentations, tests, referrals and cancer outcomes with socio-demographic factors
- Trends and patterns over time
- Highlighting of areas of inequity and opportunities where targeted interventions to level-up any observed differences may be beneficial

- Development of interventions or implementation strategies to enhance equitable access to primary care and cancer services
- Developing an understanding of the behaviours or processes involved in the primary care pathway to cancer diagnosis from the patient perspective, or the health professionals

Methods may include:

- Analysis of large quantitative datasets from primary care and linked data
- Time trends analyses; prognostic, monitoring, and prediction methodologies
- Machine learning approaches
- Qualitative research with patients and/or clinicians to develop an understanding of factors associated with differing health behaviors, healthcare and outcomes
- Co-design methodology to develop interventions or strategies targeting the general population, sub-groups of the population and/or health professionals.
- Randomised controlled trials, or emulated trials of interventions to reduce inequalities

Across all projects the candidate will be get experience of conducting patient and public involvement and engagement (PPIE).

Translational potential of the project
Understanding variation in patterns of cancer testing and referral across the NHS using large administrative datasets and conducting focussed research with communities where variation has been identified provides the basis for the development of interventions to improve rates of cancer detection and reduce inequality in cancer outcomes. Oxford is uniquely placed to conduct this work and ensure it has impact due to the unrivalled access to contemporary primary care records data and links with national charities and policymakers.

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 Cancer Theme majors in health records analysis, diagnostic reasoning, qualitative and mixed methods research in primary care, implementation science, and prospective studies of interventions to improve early detection in symptomatic patients. The NIHR Policy Research Unit for Cancer Awareness Screening and Early Diagnosis works with the Department of Health and Social Care to deliver policy relevant research to inform NHS decision making. The CPRD group specialises in the curation and analysis of primary care electronic health records data linked to the cancer registry and administrative NHS datasets.

Ideal student background: The doctoral candidate shall have a background in applied health research, social sciences, or a related discipline. Prior experience in working with quantitative data, electronic health records, or similar medical databases is highly desirable. The candidate should possess a keen interest in cancer research, particularly in early cancer detection, and have a passion for health equity. Strong communication and collaboration skills are essential, as the project involves an interdisciplinary team. The candidate should be interested in leveraging cutting-edge techniques and innovative approaches to advance cancer diagnosis. Candidates from minoritized backgrounds are encouraged to apply.

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5. Building patient-specific digital twins for cancer drug development and cancer treatment – Rachael Bashford-Rogers

Primary Supervisor: Rachael Bashford-Rogers
Additional Supervisors: Isabela Pedroza-Pacheco
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract
Cancer poses a highly multi-factorial problem, where no single dataset captures the full complexity of the tumour, the tumour microenvironment and accompanying host genetics, vasculature, lymph node and co-morbidity components that have been shown to impact patient survival and response to treatments, resulting in a highly complex problem. By capturing real-time data, the digital twin understands the current state, simulates the future state and is a basis for optimisation. It enables to detect problems early, can be used as a basis for *in silico* testing and is an opportunity to develop novel therapeutics. This project proposes to construct high-quality multi-scale cancer datasets, virtual cohorts of patients and patient digital twins which can be used for improved screening, diagnosis, clinical decision making, disease management and drug development. We will assess the short- and long-term predictive and prognostic power of such digital twins and quantify the explainable and interpretable key features underlying these predictions. Through the development of virtual clinical trials, we aim to accelerate the selection of drug combination and prioritisation in patient therapies. Overall, this may lay the foundation for more accurate and mechanistically-driven clinical decision support systems and accelerate therapeutic design in cancer.

Research objectives and proposed outcomes: A DPhil will consist of some or all of the following aspects, depending on the interests of the candidate.

Objective 1. *Develop high-quality multi-scale cancer datasets and virtual patient cohorts*. Cancer poses a highly multi-factorial problem, where no single dataset captures the full complexity of cancer. In this project, you will:

1. Bring together cancer datasets containing all -omic data types describing key features, including key genetics, transcriptomic signals, cellular neighbourhoods, cellular interactions, acellular signals, and wide organ system or whole-body signals across tumour types and corresponding healthy tissue.
2. Train different multi-layered machine learning models, with others developed through the hub, to characterise the short- and long-term predictive power, predict optimal patient treatment, provide interpretable biological learning, and identify suitable lead targets and molecules in the drug discovery process.
3. Generate virtual cohorts of patients or tumours (*i.e.* cohorts of human virtual twins) based on biological data. These realistic populations of virtual patients will be generated with the desired clinical characteristics in terms of clinical stage, grade, molecular alterations and ethnicity, sex, age and co-morbidities. Metrics will objectively assess the performance of each model in making correct predictions.
4. Leverage virtual twins together with augmented synchronous data and partial longitudinal data to reconstruct disease trajectories.

This work will be done collaboratively with experts in UCL and Sheffield.

Objective 2. *Provide biologically interpretable outputs from ex vivo experimentally-informed virtual twins.* We aim to develop global models of tumour microenvironments (TME) which could be easily streamlined into a

clinical workflow using clinical data and routinely collected clinical samples. Such models will consist of five components:

1. To identify biologically-informed feature selection from routinely collected clinical datasets learnt from higher resolution reference datasets. This may be applied to cancers where biopsies are routinely taken.
2. Clinical history data needs to be encoded and features selected, including tumour genotype.
3. Drug sensitivity experimental work for a subset of patient tumour and healthy tissue will be developed.
4. These three components could be fed into a model of the TME. This would then inform patient outcome model in which key features could be pulled out.
5. Finally, these *ex vivo* experimentally-informed virtual twins will be projected onto the models in *Objective 1* to inform and update *in silico* experiments which will be performed in the virtual clinical trials. Virtual clinical trial simulations will be parameterised to reproduce the outcome of several real trials related to cancer treatment, where responders, non-responders and partial responders may be observed.

Objective 3. *Accelerating drug discovery for targeted patient groups*. We will consider the different cancer drug classes together for accelerated drug discovery for targeted patient groups together with key novelties in each area prioritising specificity and reduction of off-target effects:

1. Chemotherapeutic agents targeting key tumour genes will be accelerated by coupling machine-learning (ML) with physics-based (PB) methods. Data generated from PB methods will be fed into ML models which are iteratively refined to generate potentially better structures. The most promising compounds will be synthesised and their thermodynamic and pharmacokinetic properties be examined, and validated in *in vitro* cell line experiments in collaboration with UCL and Sheffield.
2. Immunotherapeutic targets will be predicted and prioritised using perturbation network modelling of cell-cell communication networks derived from single cell multi-omics data. This process will be run over patient-specific tumour and germline mutational profiles, and biomarkers will be predicted for patient stratification for each prioritised molecular target.

Comparisons of the effect of the different drug approaches and combinations will be tested using the patient slide-perfusion system (outlined in *objective 2*) on relevant patient sets (collectively decided by clinicians, biologists and data scientists). Our virtual twins can be used to predict the treatment efficiency in each patient. Finally, we will apply cross-work package cutting approaches to gain insights into off-target effects of prioritised drugs/targets.

Translational Potential: This study will provide a unique platform to understand the relationship between different tumour-associated features across scales (molecular, cellular, acellular, organ systems, systemic), with the overall aim of defining improved therapeutic options and patient outcomes on a personal basis. Furthermore, the methods developed here will not just be broadly applicable to cancer, but will have wider applications in biotechnology and in health services worldwide. This will be achieved through the development and application of novel experimental and computational approaches, working in partnership with a global network of clinicians, immunologists and sample/data cohorts.

Training opportunities: The DPhil will gain experience and training in laboratory molecular biology, immunology, cancer biology, and bioinformatics. These include:

- Genomic, bulk and single-cell transcriptomic, and other ‘omic analyses across large patient cohorts.

- Development and/or implementation of novel computational pipelines for the integration of multi-scale longitudinal data with clinical covariates.
- Model building and generation of digital twins.
- The project will work in partnership with a global network of clinicians, immunologists, and computational experts.

The Bashford-Rogers laboratory has a strong track record of collaboration over the last 15 years and established systems for co-supervision.

Ideal student background: This project would be best suited for a candidate with current knowledge (or willingness to learn) both wet lab and computational skills, particularly in the fields of genetic analysis, machine learning, multi-omics data integration, however, the project can be tailored to the wishes of the candidate.

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3. Analysis of the B cell receptor repertoire in six immune-mediated diseases. (*Nature, 2019*) RJM Bashford-Rogers, L Bergamaschi, EF McKinney, *et al.*

4. Opportunities and challenges for digital twins in biomedical research: Proceedings of a workshop in brief (The National Academies Press, 2023). Linda Casola, *et al*.

6. Engineering Multicellular 3D Microtumours to Model Ovarian Cancer Minimal Residual Disease – Hagan Bayley

Primary Supervisor: Hagan Bayley
Additional Supervisors: Ahmed Ahmed
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract
Ovarian cancer is one of the most common cancers in women. Drug resistance in cancer treatment is responsible for the majority of cancer deaths. Cancer cells remaining after treatment, termed minimal residual disease (MRD), are capable of reinitiating tumours. Our understanding of MRD biology in solid tumours is limited due to the difficulty associated with isolating and characterising MRD cells from patients. Here we propose to engineer a representative 3D MRD model containing both chemotherapy resistant cancer cells and adipocytes which are known to play an important role in ovarian cancer relapse. The MRD cells will be generated from cancer organoids derived from the biopsies of ovarian cancer patients. We aim to develop a microfluidics-based method to fabricate the 3D microtumours with MRD cells surrounded by adipocytes and compatible extracellular matrix (ECM), mimicking the tumour microenvironment of MRD. We will also test the effect of different ECM materials in MRD progression. The MRD model will be used to investigate molecular pathways involved in the survival of MRD cells and screen novel therapeutics that specifically target MRD.

Research objectives and proposed outcomes
a) Background: Ovarian cancer is one of the most common cancers in women and accounts for around 4,100 deaths each year in UK.[1] Importantly, only 1 in 3 ovarian cancer patients survive over ten years. Around 70% of patients develop recurring cancer after treatment which consists of surgery and adjuvant chemotherapy. The front-line chemotherapy for ovarian cancer consists of cisplatin or carboplatin combined with paclitaxel.[1] Resistance to these drugs is common and leads to cancer relapse and mortality. Residual cancer cells that remain after clinical treatment are called minimal residual disease (MRD). MRD cells share phenotypic and genomic characteristics with the primary bulk tumour, but are capable of reinitiating tumors.[2]
Targeting MRD can prevent cancer relapse and increase the rate of long-term response. For haematological malignancies, personalised treatment of MRD has demonstrated the possibility of achieving long-term cures.[3,4] However, treating MRD in solid tumours is so far largely unexplored. Our current understanding of MRD survival mechanism is limited. Sampling MRD cells from patients with solid tumours is challenging, due to the difficulty in detecting and isolating these cells and also their scarcity. Therefore, there is a great need for building representative MRD models for both mechanistic studies and testing novel treatment strategies.

b) Project plan and previous work: Here we propose to develop a multicellular 3D MRD model derived from patients' cancer cells to i) investigate molecular pathways associated with MRD in ovarian cancer, and ii) screen novel therapeutics to eradicate MRD. We also propose that patterning MRD cells with surrounding adipocytes in Extracellular Matrix (ECM) representative of ovarian cancer will recapitulate the tumour microenvironment, which is important for ovarian cancer relapse.
Previously, the Ahmed lab performed transcriptomics analysis of biopsies from ovarian cancer patients after chemotherapy. We revealed that MRD cells share similar molecular signatures as tumour-initiating cells, expressing adipocyte-like gene signature and dependent on fatty acid oxidation (FAO) for survival and resistance to chemotherapy.[5] Further, the Bayley Lab has established a high-throughput 3D microtumour platform using microfluidics (**Fig. 1A-B**).[6] The 3D microtumours derived from cancer cell lines are able to recreate key tumour features including hypoxia that cannot be achieved using 2D cultures. The drug-resistant 3D MRD microtumours (from cancer cell lines) were able to reflect the non-genetic heterogeneity previously observed in patients' samples (**Fig. 1C**). RNA sequencing revealed that the 3D MRD microtumours resemble MRD in ovarian cancer patients with upregulated genes involved in fatty acid metabolism. We also demonstrated the use of 3D

microtumours for drug development with the identification of a promising FAO inhibitor, perhexiline, that specifically targets MRD cells (**Fig. 1D)**.

To advance and validate the clinic relevance of our 3D MRD microtumour model, as well as develop novel therapeutics against ovarian cancer MRD, we aim to: 1) Generate MRD cells from ovarian cancer organoids derived from patients' biopsies. An ovarian cancer organoids bio-bank derived from patients' primary tumours has previously been established in the Ahmed Lab. The organoids will be treated with chemotherapy drugs to generate the MRD cells. 2) Develop a microfluidic approach to fabricate patterned 3D MRD microtumours with patient-derived MRD cells surrounded by adipocytes, mimicking the MRD microenvironment in vivo. 3) Test the role of different natural ECM materials, including collagen I and hyaluronic acid, in maintaining MRD characteristics. 4) Perform molecular characterisations of the 3D MRD model through live imaging, immunofluorescent staining and RNA sequencing. 5) Test novel therapies. We will test the effect of previously discovered FAO inhibitors, drugs targeting potential new molecular pathways discovered in 4), as well as have co-cultures with T cells to test the hypothesis that drugs targeting metabolism could increase T cell response.[7,8]

We propose that this project will reveal new MRD mechanisms and discover novel treatments. The supervisors on this project have strong track records and will provide important guidance for the potential candidate.

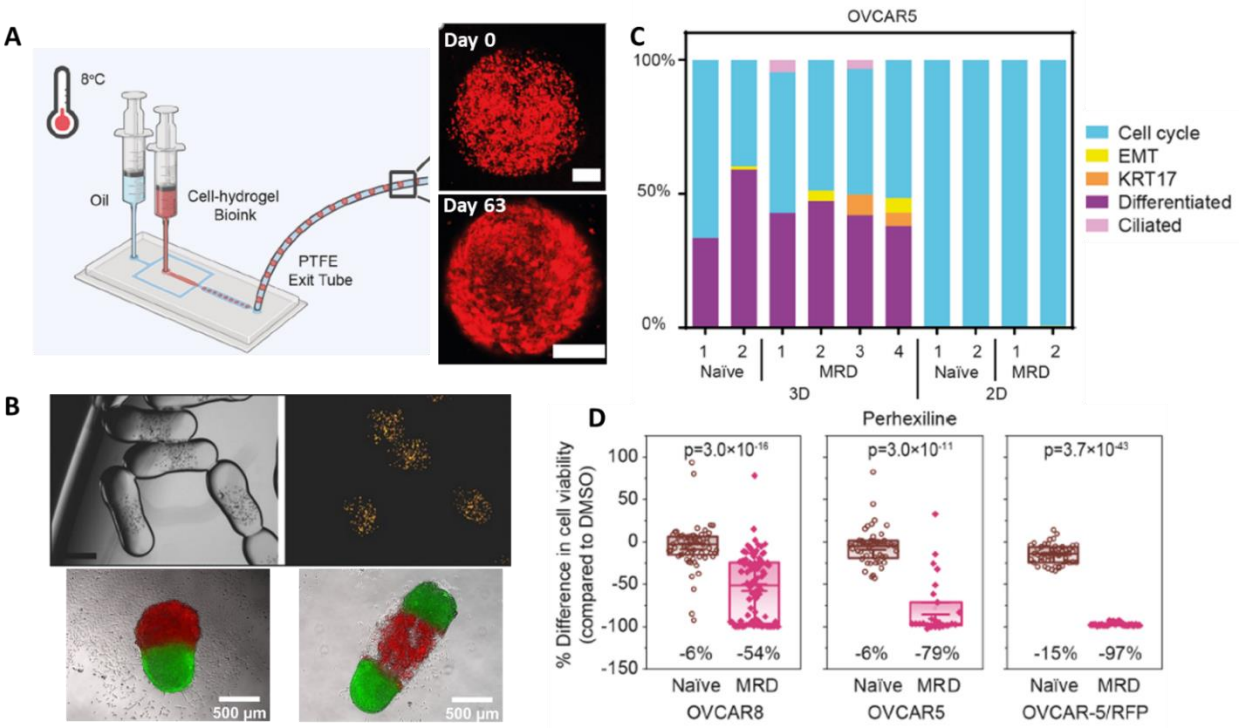

Figure 1: Microfluidic-based 3D microtumour technique and drug testing using the 3D MRD microtumours.[6] **A**, Schematic illustration of 3D microtumour fabrication by the microfluidic platform (left) and long-term culture of 3D microtumours composed of OVCAR-5/RFP (red) and Matrigel (right). Scale bars are 300 µm. **B**, Different patterned 3D microtissues from microfluidics. **C**, Percentage of cells with clinic MRD-related cell states in 3D microtumours and 2D cultures. Data from RNA sequencing result of OVCAR5 cultures treated (MRD) or non-treated (Naïve) with carboplatin. **D**, Perhexiline, a FAO inhibitor, specifically kills MRD cells in 3D microtumours.

Translational potential of the project: Drug resistance is the leading causes of cancer deaths. The proposed project would combine novel microfluidic technique and patient-derived cells to fabricate realistic 3D MRD models that is hard to achieve with organoid method. Specifically, we will advance the previously established

3D MRD microtumour platform by incorporating representative ECM and adipocytes, whose cross-talk with ovarian cancer cells has been widely documented.[9,10] This model would be used for mechanistic studies and drug discovery of MRD. The proposed research aligns with CRUK and the Oxford Centre's research priorities in developing novel therapeutics. New treatment strategies discovered in this project might be used for the treatment of ovarian cancer MRD in clinic.

Training opportunities: The potential DPhil student will be trained in the following fields: 1) Establishment and maintenance of cancer organoids and 3D microtumour cultures; 2) Adipocytes differentiation and their co-cultures with cancer cells; 3) Microfluidic fabrication and hydrogel manipulation; 4) Microscopy (especially confocal microscopy) and general molecular biology techniques; 5) RNA sequencing and data analysis; 6) Testing therapeutics using the 3D MRD microtumours.

Ideal student background: The proposed project is multidisciplinary. The potential candidate will have a background in either cancer biology, bioengineering (or another engineering field), or biochemistry. Experience in cell and organoid culture is desirable. Experience in microfluidics, hydrogels and biofabrication is favourable, but could be learned during the training process. The candidate will work collaboratively with team members engaged in cancer biology, bioengineering and polymer chemistry**.**

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7. This Project has now been removed and is no longer available for selection.

Return to Projects list

8. Applying deep topographic proteomics to solve the problem of ovarian cancer detection – Sarah Blagden

Primary Supervisor: Sarah Blagden
Additional Supervisors: Roman Fischer
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract
Ovarian cancer is the 6th most common cancer in UK and is diagnosed in 7,500 women every year. Like the majority of invasive epithelial cancers, ovarian cancer is preceded by a protracted non-invasive "precancer" phase. For example, high grade serous ovarian cancer (HGSOC) initially develops in the fallopian tube as a preinvasive serous tubal intraepithelial (STIC) lesion and, 4-6 years later, becomes invasive metastatic HGSOC[1]. However, precancers tend to be clinically "invisible" on scans or standard blood tests reflecting their different biological state. For example, whilst the circulating tumour marker CA125 can detect established HGSOC it cannot detect STICs and hence screening using CA125 has proven unsuccessful at improving survival from the disease[2]. Additionally, STICs can only be pathologically detected by microscopy, using paraffin-embedded tissue. This creates a chicken and egg situation: we have no biomarkers to detect STIC lesions pre-operatively, hence cannot remove them as fresh tissue for biological characterisation. However, using state of the art biology, we intend to conduct this research on STIC lesions that have previously been discovered within surgical specimens and archived in paraffin wax.

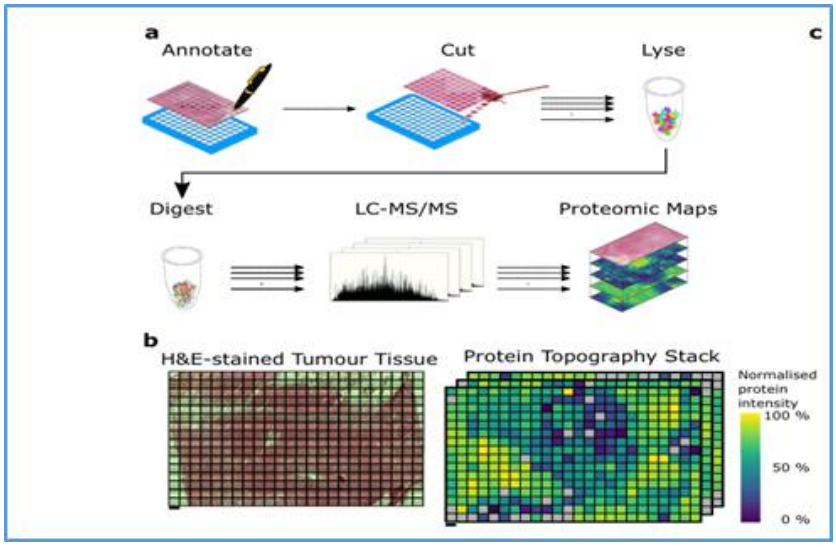
Prof Roman Fischer is the UK's foremost expert in a new technique called LCM-LCMS that combines tumour laser capture microdissection (LCM) with topographic proteomics (LC-MS), enabling detailed characterisation of over 5,000 proteins within individual cells or microscopic regions of tissue. He has optimised this technique to work on paraffin embedded specimens[3]. In this project, we will be using LCM-LCMS to characterise STIC lesions that have previously been resected during other gynaecological procedures (and tend to measure <5mm). LCM-LCMS will provide unbiased information about the proteins within STICs as well as their spatial expression. From this, we will select candidates for further validation as potential ovarian cancer biomarkers

The candidate validation stage will be done in Prof Blagden's lab which has considerable expertise in ovarian cancer research and biomarker development. Using ovarian cell lines and organoids developed in collaboration with Prof Ahmed's prestigious research group, candidate proteins will be selected and characterised using basic molecular techniques such as CRISPR, siRNA and construct-driven re-expression to determine their impact and relevance to tumorigenesis. In parallel, candidates will also be assayed in the circulation of patients with cancer – using immunoprecipitation and ELISA methods initially and then quantified in samples by Parallel reaction monitoring (PRM) mass spectroscopy.

The overarching purpose of this proposal is to characterise STIC precancerous lesions using novel microdissection and topographic proteomics from which new ovarian cancer biomarkers will be identified and validated.

Research Objectives
The project will comprise of four **work packages (WPs).**

WP1. Conduct Laser capture microdissection (LCM) using tissue blocks obtained from ovarian cancer and precancer patients along with adjacent normal tissue for comparison. Conduct liquid chromatography/mass spectroscopy (LC-MS) on excised tissue to identify candidate proteins common across all tissues as well as those unique to precancer.



Overview of the spatially-resolved proteomics workflow in WP1. ***a)*** *Tissue is segmented into a regular grid shape (Annotate), and each element of the grid is isolated by LCM into a well of a 96-well plate (Cut). Proteins from each sample are lysed in RIPA buffer (Lyse) and digested into peptides (Digest) before analysis by LC-MS/MS. The quantitative information for each protein can be mapped back to its location within the gridded tissue and visualised in a topographic protein map, with one map per protein quantified (Proteomic Maps). [figure from ref 1]*

WP2: Validate candidates by IHC using existing tissue microarrays (developed by the Translational Histopathology Lab at ORCRB) containing samples of normal, STIC lesions and HGSOC tissue. Explore the impact of CRISPR-cas9 target depletion in ovarian cancer cell lines and in ovarian organoid models.
WP3: Assay circulating plasma from normal, cancer and precancer patients for levels of identified markers using immune-precipitation/sandwich ELISA. Identify shortlist of candidates to develop PRM mass spec method for detecting constituent peptides in plasma.
WP4: Verify biomarkers in wider set of plasma samples collected longitudinally prior to ovarian cancer diagnosis.

Translational Potential
The earlier detection and prevention of cancer are central objectives of the University of Oxford and CRUK. Using cutting-edge technology, the post-holder will, for the first time, provide unbiased proteomic characterisation of STICs and take the first steps towards biomarker development for this elusive condition. Biomarkers of preinvasive ovarian cancer have enormous clinical potential, not only in detecting it when it is preventable, but also in guiding interventions such as preventive vaccines. If successful, this project could have a major transformative impact on ovarian cancer which is now considered the most lethal of gynaecological malignancy and the techniques developed here can be used to characterise other microscopic precancers.

Training Opportunities
This is an interdisciplinary project between the labs of Prof Sarah Blagden, Prof Ahmed Ahmed and Prof Roman Fischer and offers a wealth of training opportunities. The student will receive training in the cutting, preparation and mapping of specimens for laser microdissection, use of cutting-edge mass spec instruments such as Evosep, timsTOF Ultra 2 and Orbitrap Astral and basic proteomic data analysis. They will learn to maintain cancer cell lines, and organoid models alongside basic wet lab techniques. They will develop insights into biomarker development, clinical approval pathways and a comprehensive understanding of tumorigenesis biology.

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Return to Projects list

9. Understanding the origin of metaplasia – Francesco Boccellato

Primary Supervisor: Francesco Boccellato
Additional Supervisors: Jan Bornschein
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract
Metaplasia is a pathological condition where one type of mature cell is replaced by another type of mature cell that is not normal for that tissue. Metaplasia arise as a result of chronic infections or irritation and it is often considered a pre-cancerous condition. In the stomach, intestinal metaplasia (IM) is epidemiologically associated to chronic *Helicobacter pylori* infection [1] and is the best-known example of metaplasia in the gut. Patients with *Helicobacter pylori* and intestinal metaplasia are at higher risk to develop stomach cancer. Histologically, intestinal metaplasia is recognised by the detection of intestinal specific mucins in stomach histological samples. The normal mucus producing cells of the stomach are replaced with mucus producing cells that are rather typical of the lower gut. Our approach is to reveal signalling pathways involved in this tissue conversion by mapping the expression of single genes and proteins using spatial transcriptomics and proteomics. We will validate these results by pharmacological manipulation of these pathways in primary healthy stomach cells to generate metaplasia in vitro. To this end we are going to use our innovative "Mucosoids" cell culture system, a stem cell drive model which simulates the healthy stomach epithelium in vitro. This research could lead to new diagnostic tests for pre-cancerous conditions and improve early cancer detection strategies, filling a critical gap in understanding the microenvironment of pre-cancerous conditions

Research objectives and proposed outcome
We aim to generate a spatial map of the gene/protein regulatory network that underlie the changes observed in intestinal metaplasia. Our clinical collaborator Dr. Jan Bornschein is involved in the identification of patients with this condition. We will use paraffinized biopsies for spatial transcriptomic or spatial proteomics experiments. We plan map the expression of genes or proteins and we will use algorithms to understand how they interact in different positions of the tissue. The result of this analysis will suggest that specific signalling pathways are regulated differently in specific areas of the metaplasia. We aim to identify the signals triggering those pathways and to use their recombinant versions or pathways inhibitor to obtain metaplastic cells in vitro. For this purpose, we will use our patient derived advanced cell culture called the “Mucosoids”. Mucosoids are a patent pending [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] 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 signals has never been attempted. We aim to find dysregulations in specific 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 is accurate, but have a low sensitivity. A combination of ligands or proteins involved in 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. For track 1 students Clinical training will be provided by Dr. Jan Bornschein. The student will have the opportunity to learn cutting edge technologies such as spatial-transcriptomic, spatial proteomics, 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.

Ideal student background: We seek a student with a strong passion for science. For track 1 and 2 a clinical trainee in Gastroenterology or with an interest in this speciality is required.

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10. Using spatial biology and mathematical analysis to develop cellular signatures of therapeutic responses in cancer – Helen Byrne

Primary Supervisor: Helen Byrne
Additional Supervisors: Simon Leedham
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract
Tumours are complex ecosystems, in which disrupted cell interactions generate cancer cell-supportive niches, which shield tumour cells from immune surveillance and promote adaptive responses to selective pressures. Successful treatments impact key pathological cell interactions (e.g immunotherapies restore T-cell recognition to induce cancer cell engagement). Therefore, to assess therapeutic impact, we should measure tumour responses at length scales that range from the cellular to the macroscopic level. Spatial biology platforms can interrogate dynamic cellular ecosystems; however, image interpretation is a major bottleneck which requires the development and application of a new suite of mathematical tools. In this project we will establish a range of mathematical spatial descriptors that function across cell compartments and multiple length scales to characterise and quantify cellular microenvironments in pre- and post-treatment mouse cancer models. The output from these analyses will be used to define drug cellular response signatures for translation as key outcome measures in human clinical trials.

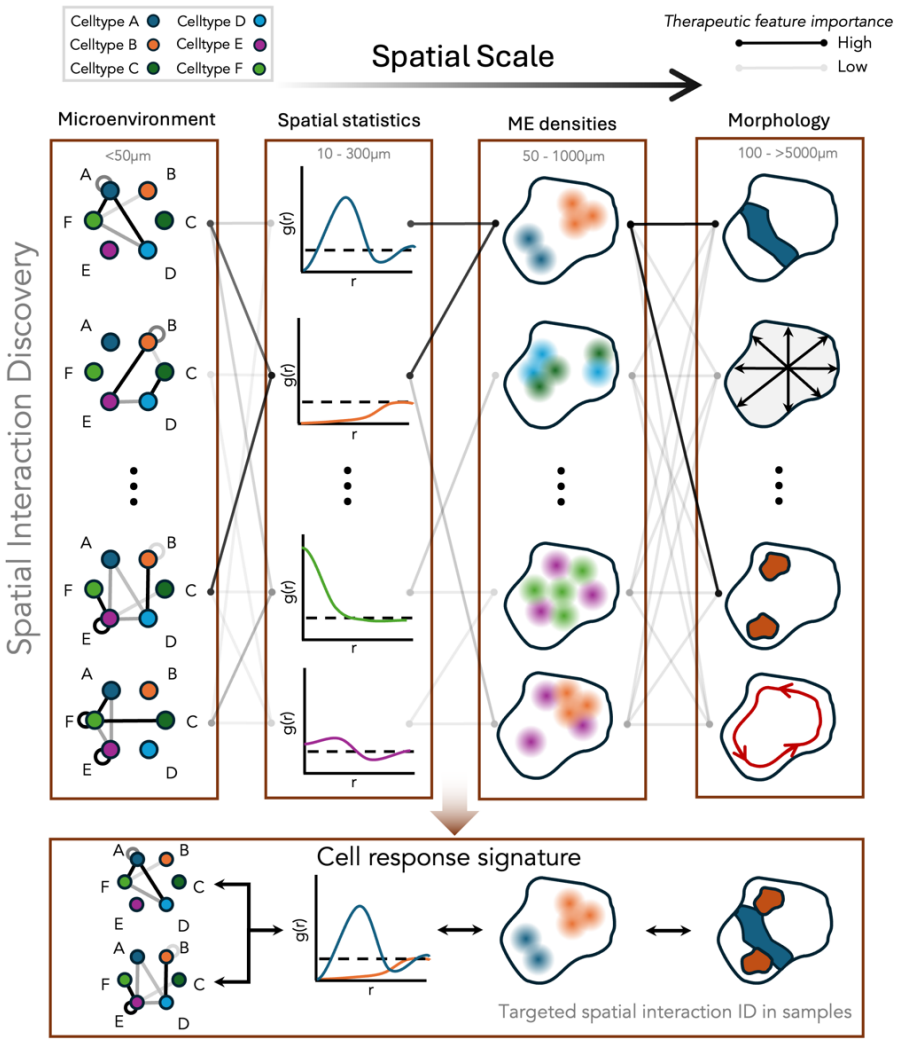

Figure 1. The development and application of multiscale spatial cellular response signatures for therapeutic quantification. Spatial features of treated and untreated samples span spatial scales from cellular microenvironments (ME) to global morphological properties and will be organised using a spatial feature hierarchy network that connects each spatial measurement to its spatially associated counterparts. The edge weights of the hierarchy network represent the importance of drug effect discrimination and will be learned using structured feature importance to extract a cellular response signature for spatial screening.

Background. Clinical trial outcomes typically rely on radiological image detection of reducing tumour volumes to define therapeutic success (RECIST criteria). This can lead to de-prioritisation of drugs that fail to impact macroscopic tumour characteristics. Some successful treatments, like immunotherapies, stimulate extensive immune cell infiltration which initially increases tumour size. In such cases, quantifying therapy response at the cellular level represents an important means of determining drug impact and titrating therapies to molecular phenotype changes.

Advancing spatial biology platforms can interrogate complex cellular ecosystems. However, the gap between our ability to generate and interpret these complex datasets is large and increasing. Since mammalian tissues are characterised by the spatial organisation of different cellular compartments across multiple length scales, it is difficult to describe them with a single metric. Some interactions are proximity-based, being mediated by direct physical contact or paracrine signalling (e.g., leukocytes with antigen presenting cells). Others act at the tissue-level (e.g., tertiary lymphoid structures at the invading edge of a cancer), and require interpretable methods that account for cell interactions that act across multiple spatial scales [1]. To address these challenges, we will apply a suite of orthogonal mathematical tools, drawn from network science, spatial statistics, ecology and topological data analysis. Each tool assesses a different aspect of complex cellular arrangements, ranging from cell counts to higher order tissue architectural structures (Fig 1). In this project, we will use this approach to identify cellular signatures of drug response in colorectal cancer.

Research objectives.
Our aim is to develop spatial cellular response signatures to emerging drugs in disease-positioned mouse models of colorectal cancer, ready for testing in human trial datasets. We will distil highly granular spatial transcriptomic datasets to identify the key drug-responsive cellular dynamics and use these to generate high-throughput companion diagnostics for application in clinical trials. In this way, we aim to catalyse a move towards the use of cellular level drug outcome response to effectively titrate therapy to impact and reduce unnecessary de-prioritisation of drugs.

WP1. Cell interaction discovery. In colorectal cancer, subtypes initiated by activation of the MAPK pathway by *KRAS/BRAF* mutation often have the worst prognosis, resulting in right sided tumours that metastasise early and seed the peritoneum. These tumours are the subject of active drug development, with new *BRAF* and *KRAS* inhibitors, used alone and in combination with downstream *MEK/ERK* therapies, currently undergoing human clinical trials. We will use disease-positioned CRC mouse models driven by activating MAPK mutations, and treated with novel *Braf* and *Kras* inhibitors to provide unlimited treated/untreated tissue for mathematical analysis. We will generate spatial transcriptomic (ST) datasets across treatments using custom mouse Xenium panels and use these discovery datasets to mathematically identify key molecular phenotypic changes induced by MAPK pathway inhibition.

WP2. Response signature definition. We will identify the key discriminatory drug-responsive cell dynamics by analysing the ST datasets from WP1 using our recently developed toolbox for multiscale spatial analysis [2], integrated within a machine learning framework [3] for structured spatial importance detection. The most discriminatory cellular microenvironments and associated spatial descriptors will be extracted and defined as cellular response signatures for a particular drug treatment (Fig 1). They will then be used to develop and optimise small, multiplex companion diagnostic panels for high throughput use (e.g Vectra Polaris).

WP3. Validation of cellular response signatures. To validate the small multiplex companion diagnostic panels from WP2, we will test therapeutic cellular responses across unseen mouse models treated with appropriate therapies, and explore cellular responses in responder and non-responder patients in appropriate human trial datasets.

Translational potential. In rectal cancer, neoadjuvant chemoradiotherapy is standard-of-care and the FOxTROT study has shown that chemotherapy in advance of surgery is safe, well tolerated and results in significant downstaging of pre-operative tumours. In microsatellite unstable disease, immunotherapies may become organ sparing as they can induce a complete pathological response. Consequently endoscopic biopsy and colorectal cancer tissue acquisition on therapy is possible and permits the development of new tools to more accurately assess tumour response to drug. Improving spatial biology techniques facilitate the assessment of dynamic cellular ecosystems and this project offers the potential to work at the forefront of the development of new clinical trial outcome metrics.

Training opportunities. The student will work within a multidisciplinary team of mathematicians, biologists and clinicians. They will learn how to analyse and interpret spatial biology sets through the development and application of established and novel mathematical tools. The project would best suit a student with a background in the biosciences, computer science, computational biology or similar discipline, who is interested in learning and applying advanced spatial biology analytical techniques.

Ideal student background: The student will have a background in the biosciences, computer science, computational biology or similar, and be keen to apply their skills to analysing biological imaging datasets.

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11. Developing an Ultrasound-mediated Treatment Modality against Osteolytic and Osteoblastic Bone Metastasis – Dario Carugo

Primary Supervisor: Dario Carugo
Additional Supervisors: Claire Edwards, Eleanor Stride and James Edwards
Eligibility: Track 2 applicants are eligible to apply for this project

Abstract
Bone metastases are highly prevalent due to the bone's high vascularity, rich mineral content, growth factor production, and intricate balance of stimulatory signalling [1]. Consequently, they commonly arise from primary breast (70%), prostate (85%), lung (40%), and multiple myeloma (MM) cancers (80%) [1,2]. Axial skeleton metastases cause reservoirs of cancer cells, destruction and weakening of bones (presenting as pathologic fractures), disability, spinal cord compression and bone pain, financial and psychosocial burden, and decreased quality of life [1]. Curative options remain scarce, with disease control achieved only over a limited time window at the expense of quality of life [3]. Bone metastases can present with osteolytic or osteoblastic mechanisms, as seen in MM and prostate cancer (PC), respectively, further complicating treatment strategies and resistance patterns. Notably, current pharmacological treatments are limited by toxic adverse effects or short half-lives to achieve clinical impact. Moreover, metastatic tumour cell dormancy, cyclical osteolytic activation, and growth hormone release hinder drug delivery and perpetuate treatment resistance. Ultrasound-responsive nanoparticles (US-NPs) offer unique capabilities in drug delivery, including enhanced drug extravasation and tissue penetration, functionalisation for targeting, and localised controlled release governed by extracorporeal ultrasound stimulation [4]. In bone, ultrasound has been successfully applied for pain palliation, either alone or in conjunction with radiotherapy. To the best of our knowledge however, the use of ultrasound-responsive drug delivery modalities in bone cancer remains largely unexplored. We hereby hypothesise that US-responsive nanoparticles can overcome the limitations of conventional pharmacological treatments by enhancing localisation and penetration efficiency of anti-cancer compounds in both osteolytic and osteoblastic bone cancer, offering the unique potential for expanding the therapeutic index of conventional therapeutics.

Research objectives
This project aims to develop an ultrasound-responsive platform to enhance and localise the delivery of anti-cancer therapeutics to osteolytic and osteoblastic bone metastases. Improved drug penetration through tissue/cell permeabilisation and controlled release from US-NPs could overcome limitations of conventional treatments associated with poor vascularisation, hostile bone marrow microenvironment, and systemic toxicity. The project will establish the safety and efficacy of this drug delivery platform by investigating mechanisms of immune activation, tumour response, and cellular stress.

Work Package 1: Identification, development and optimisation of the US-NP drug delivery platform.
Common anti-cancer and bone-targeting therapeutics will be reviewed to identify promising candidates (e.g. zoledronate, denosumab, docetaxel, bortezomib, lenalidomide) based on chemical structure, loading efficiency, clinical usage, treatment efficacy, and clinical relevance to MM and prostate-derived bone metastases. The nanoparticle will be formulated according to the selected drug's chemistry, biological target, and mechanism of action. The therapeutics will undergo bottom-up or top-down fabrication to optimise drug loading in ultrasound-responsive nanoparticulate formulations. Candidate particulate systems will include perfluorocarbon nanodroplets and gas-entrapping mesoporous silica nanoparticles. The NPs' hydrodynamic size, size dispersity, surface charge, drug loading and encapsulation efficiency, and surface morphology will be characterised. The ultrasound responsiveness of NPs will be assessed under varying US-related parameters including frequency, duty cycle, and acoustic pressure. While taxane and denosumab NPs have been developed in previous research, none have been applied in the context of ultrasound activation; therefore, this work package presents a medium risk as it aims to develop an US-NP platform for therapeutics that are rarely loaded in particulate systems [5].

Deliverables: shortlisted combinations of anti-cancer compounds and NP formulations that demonstrate sufficient stability, loading efficiency, US responsiveness, and the ability to release most of the therapeutic payload upon US exposure.

Work Package 2: Investigating the in-vitro behaviour and therapeutic potential of US-NPs.
Payload release and intracellular delivery from US-NPs (shortlisted in WP1) in osteoblastic and osteolytic monocultures will be tested in a panel of osteolytic and osteoblastic Pca and MM tumour cell lines (including PC3, C42b, ArCAPM, 5TGM1, JJN-3). The US exposure conditions used will correspond to a selected subset identified in WP1. Studies will then progress to using established co-culture models with tumour cells and bone cells (bone marrow stromal cells, osteoblasts, osteoclasts, adipocytes) [6,7]. The cell survival rate, and the half maximal inhibitory and effective concentrations will be quantified via absorbance measurements and fluorescence microscopy imaging. It is hypothesised that mechanical effects induced by NP activation by ultrasound will increase intracellular drug uptake. This will be analysed in confocal laser scanning microscopy and flow cytometry using model drugs. Drug release kinetics will be characterised via high-performance liquid chromatography (HPLC). Genomic and transcriptomic analysis of the cells will be conducted via NGS sequencing, RNA-seq and multiplex immunohistochemistry to elucidate molecular mechanisms underlying therapeutic effects induced by US-NPs. Deliverables: an optimised set of US exposure conditions that, combined with US-NPs, provide enhanced intracellular delivery of therapeutic compounds and display increased therapeutic effect on in-vitro models (when compared to control groups).

Work Package 3: Evaluating the impact of US-NPs on cellular behaviour and matrix remodelling.
After optimisation of US-NP release kinetics and delivery efficiency in monoculture and co-culture cell lines (conducted in WP2), human ex vivo three-dimensional bone models will be established and employed to evaluate the holistic effects of US-NPs on osteolytic and osteoblastic bone disease. Established PC tissue 3D cultures with conditionally reprogrammed cells have been utilised to model PC-bone cancer, while co-culture ex vivo 3D models for MM have also been successfully established [8,9]. Biomarkers associated with cellular apoptosis, cytokine expression, bone mineralisation and resorption will be evaluated via ELISA, immunohistochemistry and q-PCR. Genomic and transcriptomic analyses will be conducted, while multiplex immunohistochemistry and flow cytometry will help identify protein expression patterns and cellular interactions within the bone microenvironment. Deliverables: identification of mechanistic and pharmacological effects of US-NPs on the bone microenvironment, with a focus on markers of therapeutic efficacy, increased delivery, and safety.

Translational potential: This project aims to investigate the ability of US-responsive nanoparticles in improving the efficacy of current anti-cancer therapeutics, for the treatment of PC- and MM-derived bone cancers. The proposed research strongly complements our ongoing efforts in the development of US-activatable drug delivery systems for bone fracture healing as well as oxygen-loaded nanobubbles as a preventative strategy against bone metastases. Notably, we are currently conducting a first-in-human trial to assess localisation of US-responsive agents in bone fractures in collaboratin with the University of Southampton. These developments, concurrently with other ongoing trials evaluating US-responsive systems against glioblastoma, pancreatic and liver cancer, provide a solid basis for future translation of the US-NP formulations developed in the present study.
Training opportunities: The PhD candidate will undergo a number of training opportunities including in nanoparticle fabrication (using batch and/or microfluidic-based techniques) and chemical formulation (including functionalisation for targeting and enhanced ultrasound-responsiveness), nanoparticle characterisation (i.e., particle sizing instrumentation, electron and super-resolution microscopy), operation and modification of therapeutic ultrasound instrumentation (including clinical systems), tumour biology and immunology assays, rapid prototyping techniques (i.e., 3D printing), and development of 2D and 3D models of bone cancer (encompassing in-vitro and ex-vivo models). General research, communication, teaching, innovation, and career development skills training will be provided by the Medical Sciences Division.

Ideal student background: This project will be appropriate for candidates with a background in biomedical or biochemical engineering, bio-physics, bio-chemistry, bio-materials, or other related disciplines. Prior expertise and/or knowledge in pharmacology, microscopy, and/or particle formulation technology is desirable but not strictly required.

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12. Genetic and functional characterisation of novel immune escape mutations in DNA mismatch repair deficient (pre)cancer - David Church

Primary Supervisor: David Church
Additional Supervisors: Eleni Adamopoulou and Tim Elliott
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

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 response[1,2]. 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)[3] 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 and proposed outcomes

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 tumours, with focus on colorectal and endometrial cancers (CRC & EC) and pre-cancers
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 dMMR cancer and precancer

Preliminary data: Unpublished analysis of dMMR colorectal and endometrial cancers from the GEL 100KGP and Lynch Syndrome-associated precancers has identified high frequency recurrent frameshift mutations in immune escape candiate genes (25-50% cases). Further analysis of all 16,000 tumours indicates these mutations occur 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, TCGA (access approved) and LynchVax cohorts. In related work, the type, density, and localisation of Intratumoral immune infiltrate will be determined by multispectral co-IF (eg Vectra Polaris or Phenocycler) on

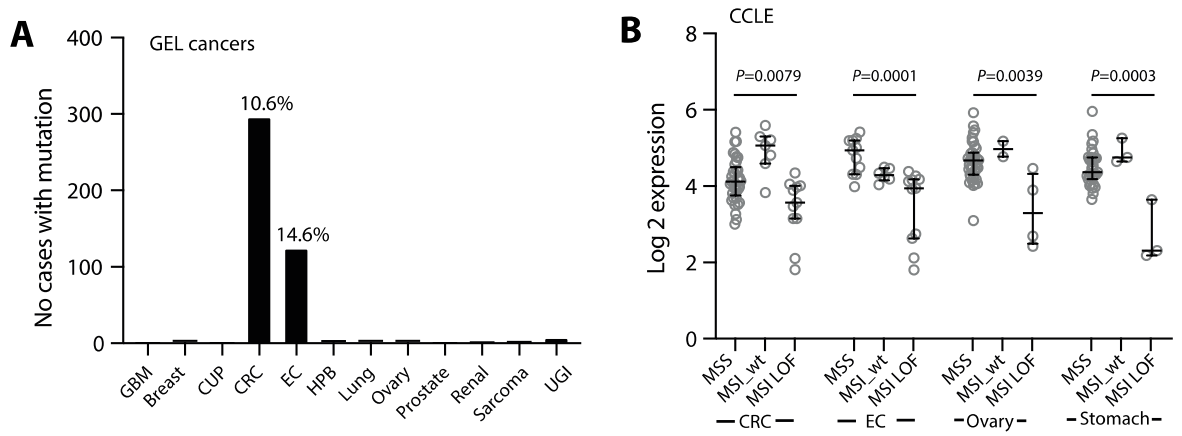

Figure 1. (A) Frequency of novel LOF immune escape mutation in Genomics England cancers by tumour type. (B) Association of MSI and immune escape gene LOF mutation status with expression in Cancer Cell Line Encyclopedia (CCLE)

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 Basel) in an extension of an existing collaboration[4,5]. 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. 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 dMMR 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 Adamopoulou 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. 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 Adamopoulou labs. If successful, experiments will be extended to human cancers and precancers and impact of LOF mutations tested in vivo in syngeneic models under immune checkpoint blockade ***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 Adamopoulou labs.

Translational potential: The widespread use of ICB for dMMRd tumours 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 our network.

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 Adamopoulou laboratories.

Ideal student background: Predominantly wet lab work but with requirement to learn and undertake bioinformatic and spatial biology analysis of data (with training and help)

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13. Benefits and Risks of Treatment Options for Hodgkin Lymphoma – David Cutter

Primary Supervisor: David Cutter
Additional Supervisors: Zhe Wang
Eligibility: Track 1 applicants are eligible to apply for this project

Hodgkin lymphoma (HL) usually has a favourable prognosis with a 10-year survival of >75% overall and >90% in people diagnosed under the age of 45 (around 50% of cases).[1] It is sensitive to a variety of treatments including conventional chemotherapy and radiotherapy (RT). Due to excellent cure rates with these treatments, the emphasis has shifted towards optimising and risk-adapting management strategies to reduce the toxicity burden of treatment whilst maintaining the chance of cure. This is of particular relevance for serious late toxicities of treatment, including second cancers and cardiovascular disease, which can be caused by both chemotherapy and RT.
Three recent randomised clinical trials (RCTs) have shown that, even when using PET-CT scans to risk-adapt treatment, the recurrence rate reduces when RT is used in the management of early stage HL.[2,3,4] A contemporary clinical challenge is therefore to balance this known benefit against the estimated risks of late toxicities from modern RT. For many patients the benefits are expected to outweigh the risks, but for a substantial minority of individuals the risks may outweigh the benefits.[5] This project aims to create new evidence that will help to meet this challenge and will be divided into two complementary components; 1) Development of an algorithm to determine the recurrence rate of HL in population-based data and 2) Development of methods to estimate the risks of RT for HL based on pre-treatment PET-CT scans.

Development of a Hodgkin lymphoma recurrence algorithm
Although the *relative* increase in recurrence risk from not using RT has been well established (the hazard ratio from a meta-analysis of the 3 RCTs was 2.77, p=0.03)[6], it is well recognised that the patients recruited to RCTs are often not representative of the whole patient population and in order to determine the *absolute* 'real world' benefit of RT it is necessary to know the absolute recurrence risks seen in a population-based cohort.

NHS England collect a wide variety of data on HL via the National Cancer Registration and Analysis Service (NCRAS) including treatment received via the National Radiotherapy Dataset (RTDS) and Systemic Anti-Cancer Therapy (SACT) Dataset. However data on recurrence are *not* routinely collected. Using the national population-based data from NHS England the student will develop an algorithm to infer the event of HL recurrence. This will be validated using regional data from Yorkshire and Humberside, in collaboration with the Haematological Malignancy Research Network (HMRN) based at the University of York, where data on recurrence is collected. The student will also work in collaboration with the National Cancer Audit Collaborating Centre (NATCAN), London where the newly established Non-Hodgkin lymphoma (NHL) audit will develop similar algorithms for NHL. Our team has previously developed an algorithm to identify breast cancer recurrence from routinely collected data demonstrating the approach is effective.[7] Information on recurrence rates among HL patient across England produced by this work will provide vital information to improve assessment of the absolute benefits of RT in HL. In addition, the methodology will be of substantial academic value, contributing to similar work in NHL and also potentially to other national audit projects currently being undertaken by NATCAN.

Estimation of the risks of radiotherapy for Hodgkin lymphoma based on pre-treatment PET-CT scans
The risk of late radiation toxicities, such as second cancers and cardiovascular toxicities can be estimated using the radiation doses received by various organs and tissues during RT, but currently this is only known with any certainty once a RT plan has been produced, i.e. when the decision to give RT as part of treatment has already been made.

The aim of this study is to develop models to predict the radiation doses received by organs and tissues during RT using information available from PET-CT scans at the time of HL diagnosis, i.e. 'upfront' when the treatment strategy is being decided. This is possible as the target volume for the eventual RT plan (if RT is given) is directly determined by the anatomical extent of disease seen on the pre-chemotherapy scan. The method will involve national and international collaboration to collate a cohort of HL patents treated with RT who had pre-chemotherapy PET-CT scans, documenting the anatomical extents of disease seen and the radiation doses received. Appropriate statistical modelling will then be used to predict radiation doses based on the anatomical extents of disease. These radiation doses can then be combined with dose-response relationships and population-based disease rates to estimate absolute excess risks of disease from RT using adaption of previously published methods.[8] The feasibility of this approach has already been demonstrated by a pilot study which showed that it was possible to predict cardiac radiation doses and risks from pre-chemotherapy PET-CT scans.[9] The principle investigator of the pilot study will act as a co-supervisor for this part of the project.

Translation Potential of the Project

The knowledge produced by this project will be of direct relevance to patients and clinicians who are making decisions regarding the best treatment options for HL. It will provide new data to help balance the proven benefit of radiotherapy against estimates of the risks from modern forms of radiotherapy. The approach will facilitate personalised management to optimise long-term outcomes for patients on an individual basis rather than following a treatment strategy based solely on PET-CT response assessment. The methods used are innovative and will be transferable to other cancer types to help progress similar ideas in other areas of cancer research.

Training Opportunities

The student will have the opportunity to study as part of a well-established multi-disciplinary team, consisting of experts in oncology, statistics, epidemiology, surgery and medical physics.[10] As part of this project the student will receive training in a variety of areas including practical research skills, radiation dosimetry, clinical epidemiology, data management and analysis.

Ideal student background: The required student will need to be a clinical trainee. Due to the radiotherapy knowledge required this would need to be a Clinical Oncology trainee.

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14. Equitably implementing improvements in cancer detection – Anna Dowrick

Primary Supervisor: Anna Dowrick
Additional Supervisors: Brian Nicholson
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract
The UK has committed to detecting 75% of cancers at stage I or II by 2028, aiming to deliver this ambitious goal using improved understanding of cancer epidemiology, advances in the development and deployment of diagnostic technologies, and the integration of decision-aids into the consultation and risk-prediction tools into the electronic medical record. Improvements promised by new technologies are rarely equally distributed among populations, and processes of generating data to support new technologies often over-represent majority populations and those who are better educated, healthier and living in urban and affluent areas. There is a risk of perpetuating barriers to diagnosis through implementing tools and technologies of cancer detection that do not reflect the needs of multiple populations. Foregrounding inequalities enables reflection on how the implementation of new initiatives can be designed to deliver greater equity in cancer diagnosis.

Despite a policy commitment to equitable implementation of improvement in cancer detection, there is limited research into the equity considerations of a range of priority cancer detection technologies: i.e. multi-cancer early detection tests (MCEDs), risk-prediction models. For example, it remains unclear how these new detection approaches might differentially impact health outcomes for ethnic minorities, people living in areas of deprivation, and people with learning disabilities. The successful candidate will be supported to develop and lead research into the equitable implementation of new technologies of cancer detection using methods that suit their intended career path.

Research objectives and proposed outcomes.
There is scope for the successful candidate to develop research objectives within the broad framework of CRUK Oxford Cancer Centre's Early Detection theme. The Early Detection theme includes a range of projects which include: evaluating the implementation innovations to improve colorectal cancer detection; optimal pathway design of MCEDs; non-specific symptom pathway optimisation. The successful candidate will be supported to develop and lead research into implementation using methods that suit their intended career path. Examples of areas for development could be to:

- collect primary data in novel implementation research designed by the candidate
- contribute original investigation within existing national implementation projects being conducted by the supervisory team.
- conduct secondary analysis of qualitative data collected from patients and healthcare professionals to explore the equity implications of early cancer detection initiatives
- expand existing implementation research projects to include a deep-dive into the experiences of minoritised groups to inform the optimal development of new cancer detection pathways
- conduct secondary analysis of existing quantitative datasets relevant to the implementation projects to see how they are dealing with race/ethnicity
- explore processes and practice leading to the membership of early detection research cohorts and biobanks to optimise diversity of inclusion

Across all projects the candidate will be get experience of conducting patient and public involvement and engagement (PPIE).

Translational potential of the project

In order for the NHS to both improve rates of cancer detection and reduce inequality in cancer outcomes it is vital to understand the factors that will ensure positive change is implemented equitably. This project will help address the implementation gap, characterising best practice in implementing improvements in cancer detection. Oxford is uniquely placed to investigate equitable implementation of cancer detection technologies as the supervisory team are involved in the development of MCED technologies and risk scores and NHS evaluations of both 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 Cancer Theme majors in implementation science, health records analysis, diagnostic reasoning, and prospective studies of interventions to improve early detection in symptomatic patients. The Medical Statistics Group specialises in quantitative diagnostic, prognostic, monitoring, and prediction methodologies, and the Medical Sociology and Health Experiences Research Group specialises in social science informed, qualitative and mixed methods implementation studies of health and illness, the NIHR Applied Research Collaboration (ARC) Oxford and Thames Valley provides support for applied health and care research that responds to the needs of local populations and health and care systems.

Ideal student background: The doctoral candidate shall have a background in applied health research, social sciences, or a related discipline. Prior experience in working with qualitative data, electronic health records, or similar medical databases is highly desirable. The candidate should possess a keen interest in cancer research, particularly in early cancer detection, and have a passion for health equity. Strong communication and collaboration skills are essential, as the project involves an interdisciplinary team. The candidate should be interested in leveraging cutting-edge techniques and innovative approaches to advance cancer diagnosis. Candidates from minoritized backgrounds are encouraged to apply.

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Return to Projects list

15. Interrogating and targeting metabolic plasticity in prostate cancer bone metastasis – Claire Edwards

Primary Supervisor: Claire Edwards
Additional Supervisors: Karl Morten
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

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.

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 (but not limited to) NBS037 and Atovaquone, 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. Advanced technology, Raman microscopy, will soon be available in NDWRH, allowing the generation of metabolic 'fingerprints' at a single-cell level, an approach we have recently combined with machine learning to develop a blood-based diagnostic test for ME/CFS (Figure 1) (7, 8). This powerful technique will significantly advance our

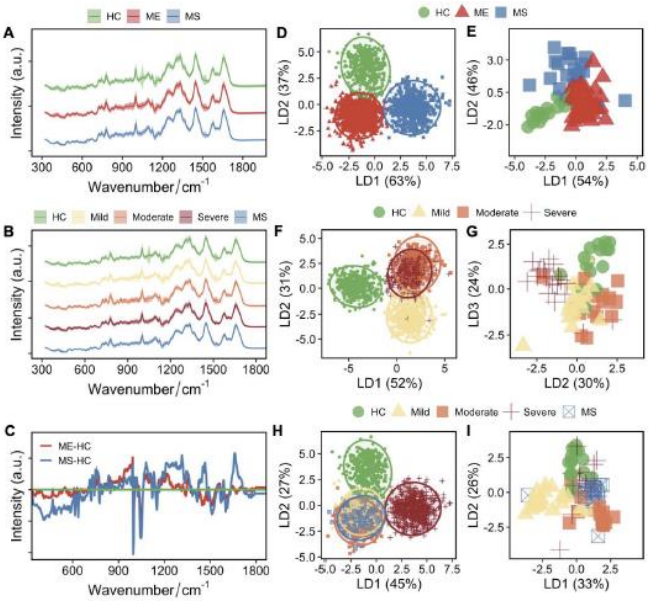

Figure 1. Single-cell raman fingerprints for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (7).

ability to interrogate and exploit the metabolic changes driving metastatic prostate cancer.

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, circulating tumour cells and bone marrow samples from patients with bone metastatic prostate cancer and from multiple myeloma. This will allow us to determine whether such metabolic signatures are disease specific or whether they may have a broader application across skeletal malignancies

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.

Supervisory Team: The supervisory team for this project draws on established biological expertise in the pre-clinical study of bone metastasis and prostate cancer metabolism (Claire Edwards https://www.nds.ox.ac.uk/team/claire-edwards) and extensive expertise in the study of mitochondria in health and disease, including targeting energy metabolism as a therapeutic strategy in cancer (Karl Morten https://www.wrh.ox.ac.uk/team/karl-morten). The studentship will underpin a new collaboration between the Edwards and Morten lab, synergising and advancing our understanding and exploitation of the metabolic relationships within the prostate cancer-bone microenvironment.

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, oncology, 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.

Ideal student background: This project will be suitable for a student with a background in biomedical sciences or medicine, or for a student with a background in physical sciences/chemistry.

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16. Investigating the link between CD8+ T cell avidity, TIL function and anti-cancer responses in oesophageal cancer – Tim Elliott

Primary Supervisor: Tim Elliott
Additional Supervisors: Felipe Galvez Cancino
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract
We have recently discovered that tumour antigen-specific tumour infiltrating lymphocytes (TIL) with low avidity are preferentially expanded in response to both Treg depletion and anti-PD1 immunotherapy and are curative (1,2). These CTL have a phenotype similar to stem-like resident memory T cells (TRM) which have been identified in a variety of different cancers including lung cancer where they have been associated with improved survival (3,4). Importantly, this population has been shown to be responsive to reinvigoration by anti-PD1 checkpoint blockade immunotherapy, unlike its terminally differentiated counterpart. They have also been observed within a limited cohort of oesophageal adenocarcinomas or OACs (5).

We have demonstrated that OACs are often highly infiltrated by a population of TRM-like PD-1+ CD39+CD103+TIM3-LAG3- antigen experienced CD8+ T lymphocytes, and that possession of a lymphocyte population enriched for this phenotype is associated with improved survival after surgery.
This project therefore aims to determine whether this T cell population in human oesophageal adenocarcinomas also have a low avidity like their counterparts in the mouse model.

Objectives and Outcomes

1. Establish a signature for low avidity, curative CD8+ T cells
Based on transcriptome data we have generated from purified low-avidity T cells isolated from *in vivo* regressing tumours (CT26 in BALB/c mice treated with anti-PD1 immunotherapeutic antibody); you will first determine the minimum number of gene transcripts that defines this population as unique. As a reference, you will use transcriptome data from high-avidity T cells that recognise the same T cell epitope but are not effective in eliminating CT26 tumours in the same way. You will cross validate your gene signature against data from a recent preprint (https://doi.org/10.21203/rs.3.rs-3903457/v1) that confirms our observation (that low avidity T cells are protective) in a second murine tumour model. Next, you will determine whether the signature you have devised is represented in human datasets where there is evidence of improved outcome as a result of CD8+ T cell activation. We have access to multiple public and in-house datasets. In particular, you will use these data to define a panel of cell-surface markers that can be used to detect and isolate specific T cell subsets from human biopsy material and blood. In the first instance, data we have generated in OAC will be used as a guide – with a view to extending the findings to other cancers. This part of the project will require basic to intermediate bioinformatic skills and training is provided as part of the DPhil programme. A rotation project in a collaborating laboratory with strong informatics would complement this project ideally.

2. Isolate CD8+ T cell subpopulations and measure avidity and functionality
Having established key transcriptional and phenotypic markers that correlate with low-avidity, curative T cells observed in mouse models, you will test the hypothesis that these (markers) can be used to isolate CD8+ tumour-infiltrating lymphocyte populations and:
a) investigate their functionality. You will disaggregate biopsies from OAC and use multiparameter flow cytometry to sort T cells into signature-positive and signature-negative populations. Bulk and single-cell RNA sequencing will be used to determine full transcriptional profiles of the sorted populations and their heterogeneity and thus an indication of their functionality – for example expression of cytokines and effector molecules, signalling molecules, and checkpoint receptors. This will also include an analysis of oligoclonality by

T cell receptor sequencing. A rotation project in a collaborating laboratory with a focus on spectral flow cytometry, cell sorting and lymphocyte cell culture would complement this part of the project ideally.
b) measure their avidity. We are fortunate to have a new technical platform in the lab to measure the avidity of cell-cell interactions, the Lumicks Z-Movi which is capable of measuring the force (in pico Newtons) required to separate T cells from their targets using an ultrasound forcefield. You will first establish short-term cell cultures (or possibly organoids) of autologous tumour cells using techniques established in Dr Parkes' laboratory, to use as target cells in the Z-Movi. A rotation project in a collaborating laboratory with primary cell culture and organoid derivation would complement this part of the project ideally. Next, you will apply purified tumour-infiltrating lymphocyte subpopulations to monolayers of target cells in the Z-MOVI reaction cell to measure their avidity. In parallel, you will clone your sorted T cell populations with a view to determining their antigen specificity, either experimentally or by inference using emerging modelling tools. Being able to recapitulate your findings relating to avidity and functionality in T cell clones with known specificity will take your investigation to another level by enabling you to dissect specific molecular signalling pathways in a tractable way. Our collaborators in NDM (Dong, Borrow, Rowland-Jones, McMichael) and UCL (Reading) have agreed to assist in this technically challenging aspect of the project.

Translational Potential
Oesophageal cancer is a major disease burden globally, with 473 000 new cases of oesophageal cancer and 436 000 deaths in 2017 and only a 10% 10yr survival rate. An understanding of the relationship between tumour infiltrating T cell avidity and T cell quality will guide the rational design of much-needed innovative immunotherapies including the selection of optimal recombinant receptors for adoptive cell therapy / CAR-TcR therapy, and the rational selection of cancer vaccine epitopes

Training Opportunities
Training in a wide range of cellular and molecular techniques using human patient samples. Quantitative biology including fundamental bioinformatic and data analytics. Multidisciplinary team working across the spectrum from molecular mechanism, computational modelling, preclinical animal models to human patient samples.

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17. Tackling cancers defective of high-fidelity DNA repair mechanisms – Fumiko Esashi

Primary Supervisor: Fumiko Esashi
Additional Supervisors: Bass Hassan
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract
Recent technological advancements in cancer genomics have revealed significant cell-to-cell heterogeneity, highlighting the role of mutability in driving cancer evolution, posing therapeutic challenges (**1**). A recent study has indicated that the simultaneous impairment of two key high-fidelity DNA repair mechanisms, homologous recombination (HR) and mismatch repair (MMR), contributes to adaptive mutability and drug resistance (**2**). Notably, while HR loss is lethal in most cell types, MMR deficiency may mitigate this lethality. Our hypothesis is that MMR-defective backgrounds enable the survival and rapid evolution of cancer cells with HR loss. To investigate the impact of MMR/HR dual deficiencies on cancer development, we propose innovative experimental and bioinformatic approaches. Specifically, by conditionally inactivating HR in MMR-defective cellular model systems, we will identify genetic and genomic factors affecting cell survival. Additionally, we will perform association analyses based on somatic cancer mutations databases to uncover potential biomarkers and therapeutic strategies for early diagnosis and treatment of these cancers.

Objectives and Outcomes
Individuals with inherited mutations within genes encoding MMR or HR factors exhibit increased risk to develop a wide range of cancers, as seen in patients with hereditary nonpolyposis colorectal cancer/Lynch syndrome (HNPCC/LS) or hereditary breast and ovarian cancer syndrome (HBOC), respectively. It is widely described that MMR defects confer mutator phenotypes with no lethal impact. Conversely, the biallelic mutations of genes encoding key HR regulators, such as the breast cancer susceptibility 2 (*BRCA2*) and the partner and localizer of BRCA2 (*PALB2*), elicits lethality, although monoallelic *BRCA2* or *PALB2* mutations are sufficient to increase cancer risk. Notably, a recent study suggests that the simultaneous impairment of MMR and HR drives adaptive mutability and drug resistance (**2**). However, the causal relationship of this phenomenon remains unclear. We hypothesise that MMR defective mutator background alleviates the lethal impact of HR loss and assists rapid evolution of cancer. This project tests this hypothesis and identifies genetic and genomic elements that are associated with MMR- and HR-defective cancers.
The genetic concept of 'synthetic lethality' or 'synthetic viability', involving the combination of mutations in multiple genes leading to cell death or growth, respectively, has gained rising attention in recent years for its potential for discovering new therapeutic targets in challenging cancers. Previous studies have relied on genome-wide loss-of-function screens in knockout cell lines. However, this approach has limitations, such as phenotypic changes obscured by secondary mutations. This project tackles these shortcomings by utilising the auxin-inducible degron (AID) technology (**3**) to conditionally deplete endogenous BRCA2 or PALB2 in MMR-defective HCT116 cell lines. This allows for highly-specific examination to uncover the direct impact of

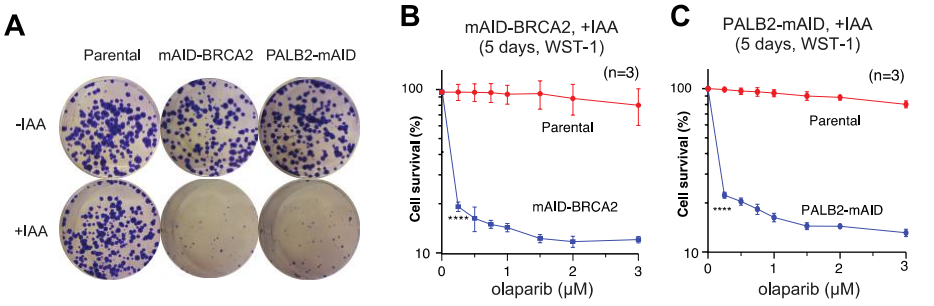

Figure 1. A. HCT116 mAID-BRCAs/PALB2-mAID cells were seeded in 6-well plates, and grown for 10 days with and without auxin (IAA). Colonies were then fixed and stained with crystal violet. **B-C:** HCT116 mAID-BRCA2/PALB2-mAID and parental HCT116 cells were first treated with IAA for 2 hours, and subsequently exposed to olaparib. After 5 days, cell survival was tested by WST assay. (n=3), error bars, SD. Asterisks indicate p value ≤ 0.0001 = ****.

BRCA2 or PALB2 depletion in MMR-defective mutator background. Our preliminary study shows that, indeed, the acute depletion of BRCA2 or PALB2 confers lethality (**Fig 1A**), as well as increased sensitivity to a chemotherapeutic drug, poly (ADP-ribose) polymerase inhibitor olaparib (**Fig 1B, C**) as expected (**4**). To identify genes that affect normal survivals of BRCA2- or PALB2-depleted HCT116 cells, we leverage the CRISPR-mediated modulation of transcription, namely **CRISPR interference/activation (CRISPRi/a)** (**5**). Our lab has already established the systems combining AID and CRISPRi/a and identified genetic factors, down- or up-regulation of which affects the survival of BRCA2- or PALB2-depleted cells. The project will characterize the cellular and molecular functions of these hits.

In parallel, we will directly assess genome changes that occur upon BRCA2- or PALB2 depletion in HCT116 cells. We will isolate several clonal HCT116 cell lines which have survived upon depletion of BRCA2 or PALB2 for one month. Our preliminary analysis indicates distinct chromosomal aberrations in these cells, arising highly repetitive centromeric regions of chromosomes. This observation is particularly intriguing as these repetitive regions are known to be targeted by MMR or HR (**6, 7**). To gain high resolution pictures of genome changes at these regions, we will conduct **long-read whole genome sequencing using Oxford Nanopore Technology (ONT)** that has advantages over traditional short-read sequencing. It enables the detection of alterations in repetitive sequences, as well as DNA modifications, such as CpG methylation. This approach is expected to provide a comprehensive understanding of the observed genome changes.

Finally, we will conduct **a bioinformatic assessment of publicly available somatic cancer mutation databases**, including COSMIC, to determine the prevalence of simultaneous impairment of MMR and HR pathways. We will initially focus colon cancers, which exhibit MMR deficiency in approximately 15% of cases. We will also explore the potential correlation between MMR/HR co-downregulation and the development of drug resistance. By examining the factors identified in our CRISPRi/a and long-read sequencing studies, we aim to uncover their association with drug resistance mechanisms. **This integrative approach will provide valuable insights into the underlying mechanisms driving drug resistance in these specific cancer types and inform the development of targeted therapeutic strategies.**

Translational potential of the project.

The proposed project holds significant translational potential. Firstly, by identifying genetic and genomic elements that influence the survival of HR- and MMR-deficient hypermutable cells, our research is expected to identify early diagnostic markers and strategies for timely intervention. We can exploit vulnerabilities specific to these cancer cells, leading to more effective treatments while minimising adverse effects. Secondly, by integrating bioinformatic analyses of cancer genomes, this project is expected to reveal the prevalence of simultaneous impairments in MMR and HR pathways in colon cancer and potentially identify previously unspecified cancer 'signatures' associated with dual HR/MMR deficiency. Further assessment of correlation between MMR/HR co-downregulation and the development of drug resistance will offer an opportunity to develop novel therapeutic strategies for these challenging-to-treat cancers. In future, similar approach could be applied to assess other types of cancers, such as ovarian cancer and pancreatic cancer, which are commonly observed in HNPCC/LS and HBOC patients.

Training opportunities

Our research project offers valuable training opportunities in key areas of cancer research, including: (1) cell culture techniques, encompassing cell line maintenance, manipulation, and experimental assays; (2) the opportunity to learn and apply long-read sequencing techniques, including sample preparation, data generation, and analysis; and (3) bioinformatic techniques for analysing publicly available somatic cancer mutation databases and exploring genetic patterns. By providing training in these areas, our project equips researchers with essential skills for future scientific endeavors in the field of cancer research. The candidates will be well supported in the Dunn school in related methods training, including CRISPR, light microscopy imaging and flow cytometry through in-house facilities, namely the Genome Engineering Oxford (led by Dr Joey Riepsaame), the Dunn School

Bioimaging Facility (led by Dr Alan Wainman), and the Don Mason Facility of Flow Cytometry (led by Dr Robert Hedley), respectively.

Ideal student background: An enthusiastic individual who has experience in research lab. Previous experience in tissue cell culture, molecular biology and/or bioinformatics analyses will be an advantage. It requires meticulous attention to detail, excellent communication skills, and the ability to develop the project in close interaction with supervisors.

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Return to Projects list

18. Integrating molecular and digital pathology to enhance prediction of breast cancer progression – Kezia Gaitskell

Primary Supervisor: Kezia Gaitskell
Additional Supervisors: Gillian Reeves
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract
Breast cancer is the most common cancer in the UK, and 1 in 7 women will develop breast cancer in their lifetime [1]. Many invasive breast cancers are thought to develop from a precursor lesion, ductal carcinoma in-situ (DCIS), but evidence is limited as to the key factors governing the progression from DCIS to invasive breast cancer. Previous epidemiological analyses in the Million Women Study cohort have investigated potentially-modifiable lifestyle risk factors for DCIS and invasive breast cancer, and suggested that BMI may be a risk factor for disease progression [2]. Other investigators have taken the approach of detailed molecular pathology analyses of a small number of cases, comparing differences in gene expression between DCIS and invasive breast cancer in isolation and in patients with both, in order to identify putative molecular drivers of progression from DCIS to invasive breast cancer [3].

In this project, we aim to triangulate epidemiological data and detailed molecular and digital pathology phenotypes from cases of in-situ and invasive breast cancer in a large prospective cohort study, to enhance our understanding and prediction of the progression from in-situ to invasive breast cancer.
The Million Women Study (MWS) is a prospective cohort of 1.3 million UK women, with detailed prospective information on anthropometric, lifestyle, and reproductive factors [4]. 90,000 cases of breast cancer have accrued after 20 years of follow-up in the cohort overall, including >6000 cases of invasive breast cancer and >800 cases of in-situ breast cancer in the Oxford area.

Germline exome sequencing data from blood samples are also available for a subset of participants in the Million Women Study cohort, including for >12,000 women with invasive breast cancer, >2000 women with DCIS alone, and >500 with both. Of these, approximately 400 women with invasive breast cancer and 60 women with DCIS alone are from the Oxford area, for which tissue samples may be available for molecular and digital pathology analyses.

In this project, exome sequencing data from the sub-cohort will be used to develop a common- and rare-variant genetic prediction model for progression from DCIS to invasive breast cancer. The key germline genetic predictors identified in these analyses will then be taken forward for further molecular testing and validation in the subset of cases with tissue samples in Oxford.

We aim to retrieve archival histopathology slides and tissue blocks from selected cases of in-situ and invasive breast cancer in MWS participants in Oxford, and perform detailed digital and molecular pathology analyses. This will include scanning the glass slides to generate digital whole-slide images, which can be analysed using AI/ machine-learning techniques, and performing molecular characterisation on tissue blocks.
Spatial molecular techniques (e.g. spatial transcriptomics/ proteomics) will be used to investigate whether these key germline genetic variants, identified from exome sequencing analyses, correspond to tissue-specific variation in gene/ protein/ mRNA expression, comparing between normal background breast tissue vs in-situ vs invasive breast cancer. Other molecular differences between in-situ vs invasive breast cancer will also be investigated. Digital pathology image analysis and AI/ machine-learning techniques will also be used to explore imaging-based correlates of these molecular changes. Alternative and/or additional analyses will also be considered and developed in consultation with the student.

This combination of large-scale epidemiological risk factor data on the whole cohort, with rich genomic and digital and molecular pathology data on a subset of cases, will provide an unparalleled resource to investigate the biological mechanisms underlying the progression from in-situ to invasive breast cancer, with important clinical applications for prognostication and planning treatment.

Research objectives:

1. Develop a common- and rare-variant genetic prediction model for progression from DCIS to invasive breast cancer, using exome sequencing data from blood samples from MWS participants with in-situ and invasive breast cancer.
2. Characterise the molecular profiles of selected breast cancers (including invasive, in situ, and those with both in situ and invasive disease present) among MWS participants with archived tumour tissue samples available in Oxford, based on immunohistochemical markers (ER, HER2, PR, Ki67, androgen receptor status), and spatial 'omics' analysis of archival tissue samples, and compare these profiles according to the co-existence of in situ and invasive disease. Explore whether the genetic variants identified from exome sequencing analyses correspond to expression differences in tumour tissue.
3. Validate existing machine learning (ML) based algorithms for prediction of molecular subtype and other molecular biomarkers based on digitised pathology slides in these patients, and compare their performance with that from a novel ML based algorithm derived from these data.
4. Develop a novel algorithm based on information from digital slides, immunohistochemical, and other multi-omic biomarkers for predicting the probability of progression from in-situ to invasive disease, and of death from invasive disease.

Proposed Outcomes:

1. This project will generate a common- and rare-variant genetic prediction model for the progression from DCIS to invasive breast cancer.
2. This project will provide important new information on detailed molecular profiles of both in situ and invasive breast cancers in a relatively representive sample of UK breast cancer patients aged 50 and over, including information on comparatively novel markers such as androgen status, the prevalence and prognostic significance of which is still unclear.
3. The analysis of tumour molecular profiles according to the degree to which invasive disease occurs alongside in situ disease will help identify those in-situ cancer patients who are most likely to progress to invasive disease.
4. The project will enable independent validation of published ML based algorithms for prediction of molecular subtype using digital slides in a large population-based sample of NHS patients.
5. The project will generate and assess the accuracy of a comprehensive algorithm, including immunohistochemical and molecular markers together with digital pathology, for predicting outcome for both in situ and invasive breast cancers
6. The project will help develop important new collaborations between clinical pathologists, epidemiologists, data scientists and engineers, aimed at exploiting existing data from large population-based studies to address major outstanding questions about the potential value of digital pathology for routine subtype classification and predicting disease outcome

Translational potential

This project has a high potential for clinical translation and enhancing patient care. Improved genetic prediction, and characterisation and validation of digital and molecular pathology markers, of progression from in-situ to invasive breast cancer could inform prognostication and shared decision-making on planning treatment – both for identifying patients with a high risk of disease progression, who may require more aggressive treatment, and also for those with a low risk of progression, for whom less-aggressive treatment may be an option.

Training opportunities

As part of this project, the student will receive comprehensive training in epidemiology, statistical analysis of complex linked health records data, analysis of sequencing data, molecular and digital pathology, and machine-learning methods as applied to digital pathology. There will also be opportunities for developing communications skills through presenting results at scientific conferences, writing manuscripts for publication, and contributing to public engagement activities.

Ideal student background: The selected individual for this project should have strong quantitative skills, preferably including familiarity with either epidemiology and statistics and/ or machine-learning methods. Experience of digital pathology would be advantageous but not essential. They will join a diverse team, gaining expertise in epidemiology, statistical modelling, and machine learning, through comprehensive computational training. The role includes opportunities to present findings at international conferences.

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19. Deciphering the CD8+ T cell landscape and reactivity during the development of hepatocellular carcinoma - Felipe Galvez-Cancino

Primary Supervisor: Felipe Galvez-Cancino
Additional Supervisors: Ellie Barnes and Tamsin Cargill
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract
In the UK, liver cancer (Hepatocellular Carcinoma, HCC) is one of the few cancer types increasing in both incidence and mortality in the UK and globally [1]. $CD8^+$ T cells are key players during liver inflammation [2], and during the response to immune checkpoint blockade in HCC [3]. However, the changes in the $CD8^+$ T cell landscape and the antigens these cells recognise during the transition from inflammatory liver disease to advanced fibrosis and HCC remain poorly characterized [4]. $CD8^+$ T cells have both pathogenic roles (e.g. in non-alcoholic steatohepatitis) but also protective (e.g. against chemically induced liver fibrosis and HCC) [2, 3, 5]. It is feasible that pathogenic and non-pathogenic responses are driven by different antigens within the liver niche. In this context, identifying antigens that can promote an effective anti-tumour $CD8^+$ T cell response can potentially be exploited for developing preventive cancer vaccines. Furthermore, the specific inhibitory and costimulatory molecules regulating $CD8^+$ T cell activity as well as their crosstalk with the surrounding microenvironment, like myeloid and Treg cells hold the key for the identification of novel targetable axes. A better understanding of these cell-cell interactions will inform the discovery of antigens and new therapeutic targets for vaccine and therapeutic antibody development in liver disease. This proposal aims to understand the key aspects of immune regulation during the transition from inflammatory liver disease to HCC. We aim to characterise the antigen-specific $CD8^+$ T cell response and its intrinsic and extrinsic regulation within the liver microenvironment.

Objectives and Outcomes
1. Understand the changes in antigen specific CD8+ T cell responses during the progression from inflammatory liver disease to HCC.
You will explore antigen specific $CD8^+$ T cell responses that occur during the transition from inflammatory liver disease to HCC, identifying common antigens and reactive T cell receptors that become present during this transition. You will use bioinformatic tools to predict peptides from common antigens and commonly mutated proteins that have been frequently identified in patients with HCC and liver disease (such as α-Fetoprotein, Glipican-3, NY-ESO-1, MAGE-A, P53, etc). These peptides will be used in immune assay (IFN-g ELISpot and Flow Cytometry) to stimulate antigen specific T cells in blood and tumour tissues obtained from patients with inflammatory liver diseases and early HCC. T cell receptor cloning will identify reactive T cells and their cognate T cell receptors. It is expected that the identification of antigens common to different patients will allow for the development of new preventive cancer vaccines for patients at risk of developing HCC. Furthermore, the identification of relevant T cell receptors has the potential for the development of new cell therapies.

2. Identify intrinsic and extrinsic immune-regulatory pathways controlling the activity of CD8+ T cells within the liver microenvironment.
The presence of tumor-specific $CD8^+$ T cells within tumors demonstrates that despite their presence there are immune-regulatory pathways restraining their activity. The blockade of the PD1-PDL1 axis in HCC has shown therapeutic efficacy and is now standard of care treatment, demonstrating that T cell responses can be leveraged by blocking specific checkpoints. However, the regulatory mechanisms that control T cell responses during the early phases of tumor initiation in HCC remain unknown. Using the previously identified T cell receptors in Aim 1 you will use single cell RNA sequencing to identify these T cells, understand their phenotype and the specific checkpoints that they express. We expect that these analyses will lead to the identification of

novel therapeutic axes that can be therapeutically exploited using monoclonal antibodies or small molecule inhibitors. Furthermore, by combining the single cell RNA sequencing data with spatial transcriptomics (Xenium platform) we expect to understand the extrinsic regulation of antigen-specific $CD8^+$ T cells potentially mediated by interactions with suppressive cells like regulatory CD4+ T cells and myeloid cells. This part of the project is complimentary with Aim 1 and has a strong focus on data analysis.

3. Assess the changes in T cell response and T cell reactivity following standard following immune-checkpoint blockade

The previous two aims will lead to identification of antigen-specific $CD8^+$ T cells and their intrinsic (checkpoints/costimulatory molecules) and extrinsic regulation (regulatory $CD4^+$ T cells/myeloid cells). The Galvez-Cancino lab has adapted a patient-derived tissue fragment platform that allows for the culture of small tumour fragments that can be perturbed in the presence of therapeutic antibodies and small molecules. Based on the discoveries of Aim 1 and 2, you will have identified antigen-reactive CD8+ T cells and regulatory mechanisms that can be perturbed to increase their activity. Using tools available in our lab (anti-PD1/anti-CTLA4/anti-CD25) you will assess whether checkpoint blockade, regulatory $CD4^+$ T cell depletion or the perturbation of myeloid cells can increase the activity of tumor-reactive $CD8^+$ T cells.

Translational Potential

Primary liver cancers (hepatocellular carcinoma [HCC] and intrahepatic cholangiocarcinoma) are the fourth leading cause of malignancy-related mortality worldwide. Curative therapy with tumour ablation, resection or liver transplantation is dependent on the early detection of HCC (EDx). However, 70-80% of HCCs are diagnosed at a late stage when treatment is futile (overall 5-year survival <5%). Therefore, effective strategies for HCC-EDx are urgently required. There is a lack of knowledge regarding the biological pathways that drive the transition from inflammatory liver disease to HCC. Understanding this in depth would allow us to develop new strategies for HCC EDx, as well as treatment and even prevention

Training Opportunities

This project is the result of a new collaboration between cancer immunologists, vaccinologists and clinician scientists who are seeking to establish Oxford as a Centre of Excellence in Liver Cancer, and to build collaborations between the Vaccine and Cancer themes in the Oxford NIHR BRC. The project will give an outstanding opportunity to work on human samples, with translational potential, using state of the art techniques for immune assessment at cellular/protein, transcriptomic and spatial resolutions. Key techniques that will be applied in the project include IFN-gamma ELISpot, Multi-parametric Flow cytometry, single cell RNA sequencing, spatial transcriptomics and bioinformatics.

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20. Relationship between Tumour microenvironment and atherosclerotic cardiovascular disease during immunotherapy - Audrey Gérard

Primary Supervisor: Audrey Gérard
Additional Supervisors: Claudia Monaco
Eligibility: Track 1 applicants are eligible to apply for this project

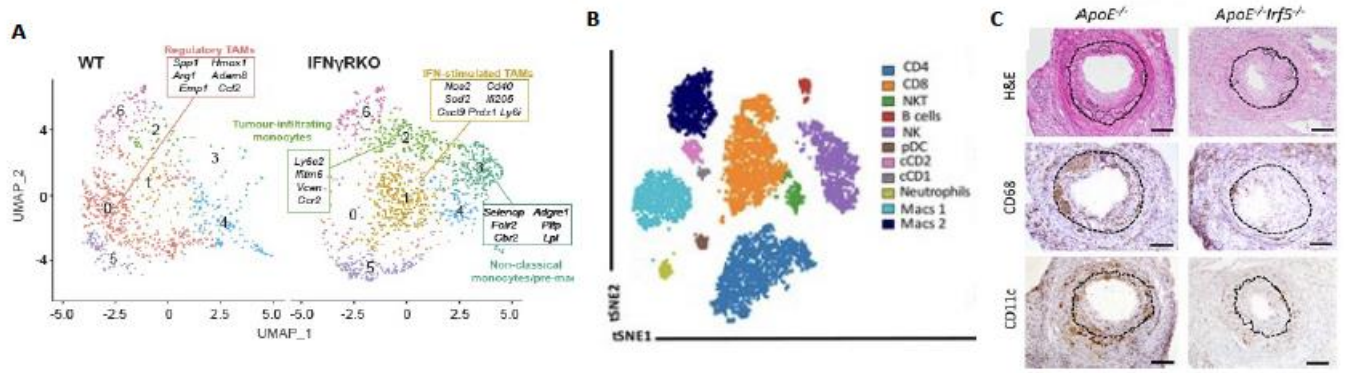
Abstract
Cancer cells can be recognised and killed by our immune system. However, tumours developed strategies to evade the immune system by rewiring immune cell types that are critical for tumour control. In recent years, therapeutics such as immune checkpoint inhibitors emerged to repristinate immune monitoring, and they indeed have shown unprecedented success in treating aggressive cancers. The widespread clinical use of immune checkpoint blockade (ICB) has increased our knowledge on their adverse effects on chronic inflammatory diseases. Among those, atherosclerosis, a low-grade lipid-driven inflammatory disease of the larger arteries, is commonly present in cancer patients. Cancer patients receiving ICB have an increased risk for atherosclerotic cardiovascular disease (CVD). This project aims at understanding the relationship between the tumour microenvironment, the atherosclerotic immune landscape and how they are affected by ICB.

Research objectives and proposed outcomes
Tumours actively escape the immune system by inducing an immunosuppressive state where intra-tumoural CD8 T-cells are "exhausted", expressing an array of checkpoint inhibitory receptors, which results in a lack of effector functions. Immune Checkpoint Blockade (ICB) aims at reinvigorating the immune system and those exhausted T-cells to control tumors by blocking those inhibitory receptors[1]. However, the response rates are still only 15 to 30%, in part because tumours have developed escape strategies to evade checkpoint blockade. In addition, patients treated with immune checkpoint inhibitors had a higher risk of CVD events. Within six months of starting treatment, patients with lung cancer on PD1 inhibitors had double the risk of CVD events; malignant melanoma patients had a 4.3-fold increased risk if they were being treated with PD1 inhibitors and a nearly five-fold risk if they were receiving the CTLA-4 inhibitor[2]. The adverse effect of ICB on atherosclerosis-related cardiovascular disease is therefore a major concern, resulting in cardiovascular events, such as myocardial infarction or ischaemic stroke[3].

The effects of ICB on atherosclerosis in cancer patients are incompletely understood. Tumour immunity relies on immune mediators, such as the cytokine IFNγ. Following ICB, rejuvenated CD8 T-cells start producing vast amount of IFNγ. IFNγ-related gene signature is a predictive marker for immunotherapy efficiency in multiple tumour types[4]. Mechanistically, IFNγ inhibits both tumour proliferation, as well as angiogenesis and has pro-apoptotic effects on cancer cells. As such, IFNγ is inherently linked to the efficacy of tumour immunity. In the other hand, IFNγ is highly expressed in atherosclerotic lesions and has emerged as a significant factor in atherogenesis[5]. Overall, IFNγ drives an inflammatory immune response (Th1, M1 macrophages…) which is beneficial to anti-tumour immunity, but likely detrimental to atherosclerosis.

As such, it is important to understand how ICB integrates with the tumour microenvironment (TME) and the immune landscape of atherosclerotic plaques. In order to do this, we need to establish the relationship between the TME and the atherosclerotic plaque immune landscape. It had recently been shown that the immune landscape elicited in tumours is accompanied by a systemic effect on immune cells initiated in the bone marrow[6]. Therefore, we hypothesise that tumours with different TMEs may skew atherosclerosic plaque immune landscape.

We will use a model of atherosclerosis whereby atherosclerosis-prone mice (ApoE−/−) will be fed with a high-fat diet (HFD). Those mice will be engrafted with multiple tumor cell lines which generate a different TME (example in Fig.1A). We will analyse the TME, vascular and systemic immune landscape, with a particular interest in macrophages (Fig.1B,C), because they are crucial for tumour and vascular immunity, and their differentiation is regulated by IFNγ. To do so, we will use imaging, flow cytometry and CYTOF. We will then deplete macrophage subsets that are present in one or both sites and analyse tumour growth in the presence or absence of ICB.



Immune landscape in tumours and atherosclerotic plaques. A- Transcriptomics analysis of the myeloid compartment of 2 tumour types (WT and IFNγRKO B16 melanoma tumour cells) that generate distinct immune/myeloid landscapes, with macrophage subset markers[7]. B- Representative CYTOF analysis of a human carotid plaque with tSNE plots of macrophage and dendritic cell markers in human atheroma cells[8]. This shows that The majority of CD11c+ cells in human carotid plaques are macrophages. C- Representative images of cross-sections of carotid arteries from ApoE-/- mice on HFD. The sections were stained with haematoxylin and eosin for plaque and necrotic core size (necrotic cores are indicated with a dotted line). CD68 and CD11c were used to identify macrophages.

Research Objectives:

i) Characterise how tumours with distinct TMEs influence the systemic immune landscape and the immune landscape of atherosclerotic plaques. We will focus on different macrophage subsets and investigate their role in both the TME and atherosclerosis plaques.

ii) Determine whether the TME influences the severity of artherosclerosis. For this, we will analyse the frequency of plaque rupture and reduced both necrotic core and CD11c plaque area in an inducible plaque rupture model.

iii) Explore the effect of immunotherapy on the TME and its relationship with the immune landscape in atherosclerotic plaques and the effect on the severity of artherosclerosis.

Outcome: This project will unravel the relationship between the tumur microenvironment and the risk of atherosclerosis, both at steady state and during immunotherapy. We hypothesise that immune checkpoint proteins orchestrate the inflammatory response underlying atherogenesis. This will uncover potentially targetable new mechanisms to tackle this type of co-morbidity.

Translational potential of the project.
This project will help us understand how tumours and their microenvironment influence the atherosclerotic immune landscape. Optimal cardiovascular risk management in ICB-treated patients is opportune to reduce the occurrence of cardiovascular disease in cancer patients and long-term cancer survivors. This project might help advising on this.

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. This project provides broad training in cancer biology and immunology covering a range of cellular, molecular and functional immune assays. Students have access to cutting-edge technologies such as disease mouse models of cancer, multiplex imaging, spectral flow cytometry, CYTOF. The Gérard Lab and Monaco Lab have complementary skills in tumour and vascular immunology, and are highly collaborative. There is extensive technical support in all techniques required for this project and both labs provide a supportive environment in which PhD students thrive.

Ideal student background: The student requires basic knowledge in immunology. Knowledge in tumour or cardiovascular immunology is a plus. Experience in immune assays, in vivo or ex vivo is necessary. In addition, the student should be willing to work with animal models.

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21. Machine perfused hemi-livers to study metastasis immunobiology – Alex Gordon Weeks

Primary Supervisor: Alex Gordon Weeks
Additional Supervisors: Kerry Fisher
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract

Current experimental models fail to mirror the complex heterogeneity of human cancer and are limited in their ability to physiologically profile viral and cell-based therapy delivery. We have developed a fully human liver cancer platform encompassing extended duration (72 h) normothermic perfusion of hemi-liver specimens from cancer patients. We perform serial perfusate, healthy liver and liver tumour analysis to generate an unparalleled understanding of the immune contexture of liver tumours and their response to therapies.

To date, we have perfused 25 hemi-liver specimens containing either primary or metastatic cancers. We demonstrate liver functional stability through continuous physiology and biochemical monitoring. This technology, has demonstrated, for the first time, oncolytic virus replication in the cancer (Fig.1) and failure of immune cell extravasation in tumour relative to healthy liver (Fig 2).

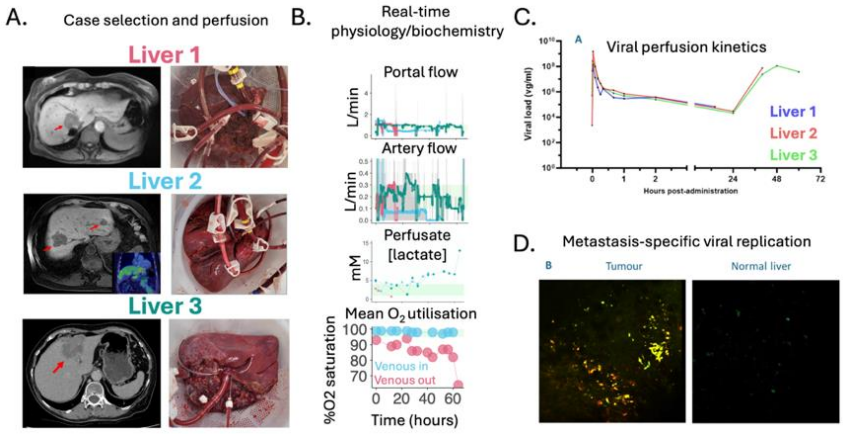

Fig. 1. Tracking oncolytic virus infectivity in-vivo. **A)** selection of three perfused hemi-livers containing cholangiocarcinoma (liver 1) and CRLM (livers 2-3) based on pre-operative imaging. **B)** Real-time physiological and biochemical monitoring of the perfused livers over a 60-hour period. **C)** Circulating oncolytic virus kinetics following injection into the perfusate. **D)** immunofluorescent detection of infected cells (red) and replicating virus (green) in the liver tumours and normal liver at the end of the experiment.

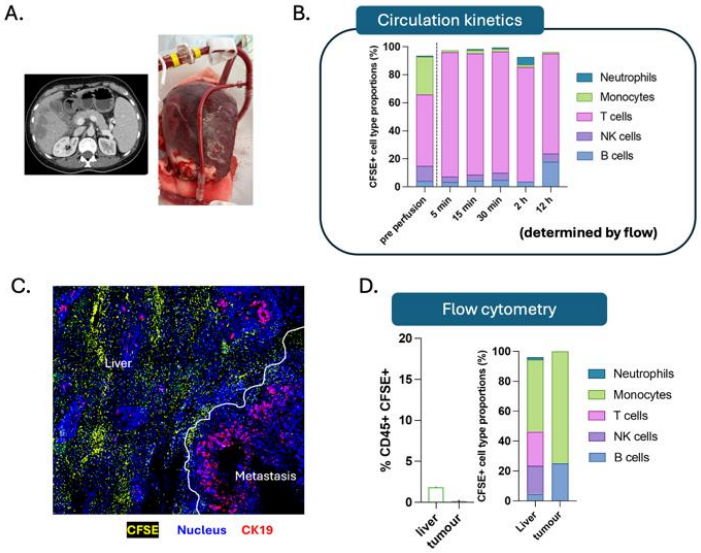

Fig. 2. Immune cell delivery to perfused liver tumours. **A)** selection of a liver containing CRLM stably perfused with packed red cells. **B)** FACS quantification of immune cell populations from a CFSE-labelled buffy coat before and then from serial perfusion samples following injection into the perfusate. **C)** fluorescence IHC demonstrating extravasation of the CFSE-labelled immune cells in the liver but not the CRLM at the end of the experiment. **D)** FACS quantification of the number of CFSE-labelled immune cells in the liver tissue and CRLM and their composition at the end of the perfusion.

Here, we will use hemi-livers with primary or metastatic liver cancer to study the mechanisms that govern failure of T-cell extravasation within the TME. We will track immune cells as they leave the perfusate and extravasate into the liver and liver cancers, and study the mechanisms through which T-cells fail to extravasate into the tumour. Subsequently, autologous Chimeric Antigen Receptor (CAR)-T-cells targeting EPCAM on the cancer cell surface, will be infused alongside agents to increase T-cell tumour extravasation (TNF, bi-specific antibodies targeting immunosuppressive fibroblasts, US-mediated cavitation). Findings will be directly translatable through window of opportunity trials in liver cancer patients.

Research Objectives and Outcomes.

Both primary and metastatic liver cancers have a poor clinical outcome with relatively limited therapeutic options. Liver surgery offers the best chance of long-term cure but is an option for few patients. Cancers in the liver are particularly insensitive to immunotherapeutics. The mechanisms behind this may relate to failed T-cell extravasation within the cancer and T-cell exhaustion following extravasation. These mechanisms are documented in replacement and desmoplastic CRLM, which demonstrate few T-cells or T-cell exhaustion respectively (Fig 3) but have not been studied in living cancers.

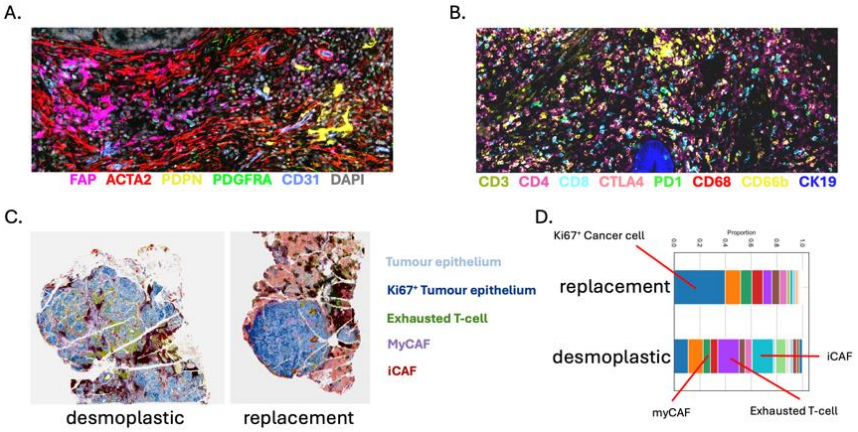

Fig 3. Multiplexed spatial characterisation of CRLM subtypes. A-B) multiplexed panels stained using CellDIVE. **C)** Scimap spatial analysis demonstrating significant heterogeneity between desmoplastic and replacement subtypes. **D)** Cellular neighbourhood differences between CRLM subtypes.

The project is divided into two **primary objectives**:

1. **To investigate immune cell trafficking within normal liver and liver cancers**. Perfused cancer-containing hemi-livers are dosed with fluorescently-labelled immune cells. ***Outcomes.*** Serial samples of the perfusate, liver and liver tumours are profiled using FACS to enumerate immune cell types kinetically. Multiplexed immunohistochemistry (CellDIVE, MIBI) is performed on serial tumour biopsies to monitor spatial trafficking of immune cells in the TME over time.
2. **To investigate methods to improve T-cell extravasation within the TME**. Autologous CAR T-cells targeting EPCAM are developed pre-operatively in patients undergoing hemi-hepatectomy. These are administered alongside (or without) treatments to improve T-cell extravasation including TNFα and bi-specific antibodies targeting fibroblast subtypes. ***Outcomes***. Comparison of CAR T-cell mediated killing in treated and untreated tumours using FACS and multiplexed spatial analyses.

Translational Potential.

This disease model has an unparalleled ability to represent several features of human liver cancers not currently afforded by any other model system. These include the full complement of tumour microenvironmental cell types, delivery of oxygen and nutrients at physiological pressures and concentrations and mapping of cellular responses to treatment over time. Because the model can mimic all subtypes of human liver cancer, we can infer cancer subtype-specific therapeutic responses such as growth pattern-specific responses (Fig 3). These features will lead directly to a personalised approach to trial design and a de-risking of early-phase therapeutic studies which we are well placed to carry out subsequently in Oxford.

Training opportunities.
These are broad and can be tailored to the applicant's specific career aims. They range from the ability to integrate within the institute of biomedical engineering focusing of further model development, through understanding of PK and sample analysis utilising a range of techniques including RNA-seq (bulk and single-cell) and spatial approaches (Cell-DIVE multiplexed IHC and Multi-Ion Beam Imaging) as per figure 3. The applicant will also be supported to develop capabilities in multicellular organotypic culture techniques; supporting findings from the liver perfusion platform.

Ideal student background: An interest in translating basic science directly into clinical practice. Experience in any field of cancer research but would be most suitable for someone with a practical temperament given the role of engineering in the experimental model.

Return to Projects list

22. Understanding variable clinical outcome in paediatric brain tumours – James Grist

Primary Supervisor: James Grist
Additional Supervisors: Ester Hammond
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract
Brain tumours are one of the most common oncological causes of mortality in the adult and paediatric population. Dr Grist has previously developed an AI-based model using Magnetic Resonance Imaging (MRI) that can identify children with tumours that are highly lethal, leading to a much earlier death than would be expected for that type/grade, linked to abnormal blood supply [1]. Interestingly, this model can identify individual patients with lethal tumours that would traditionally be considered 'low risk' (e.g. pilocytic astrocytoma) and vice-versa. **This project will aim to take these results to the next level and validate the model in a prospective study, as well as understand the underlying tumour biology leading to lethal tumours.** If successful, this project could provide novel therapeutic targets, as well as identify those children that may benefit from inclusion in studies with more experimental therapeutic approaches.

Due to the rarity of paediatric tumours, a UK-wide tumour network, the Children's Cancer and Leukaemia Group (CCLG), has been in place for over 25 years and, through the work of Dr Grist and Dr Wilson, Oxford is now a member contributing to this network. Our working hypothesis is that this decreased blood supply leads to a hypoxic state within the tumour, which in turn pushes the cells toward an aggressive phenotype[2].
We would like to offer the exciting opportunity for a DPhil student to drive collaborative study to understand the underlying biology of this hypoxia phenomenon, and to assess whether the blood supply data hold up in a prospective assessment in children across the UK CCLG network. Oxford is a paediatric neuroncology centre and sees most brain tumour cases in the region. In turn, this gives us the opportunity to capture imaging and tissue from a wide range of tumour types and grades to enable the DPhil, we already have ethics in place for the project.

Research objectives and proposed outcomes

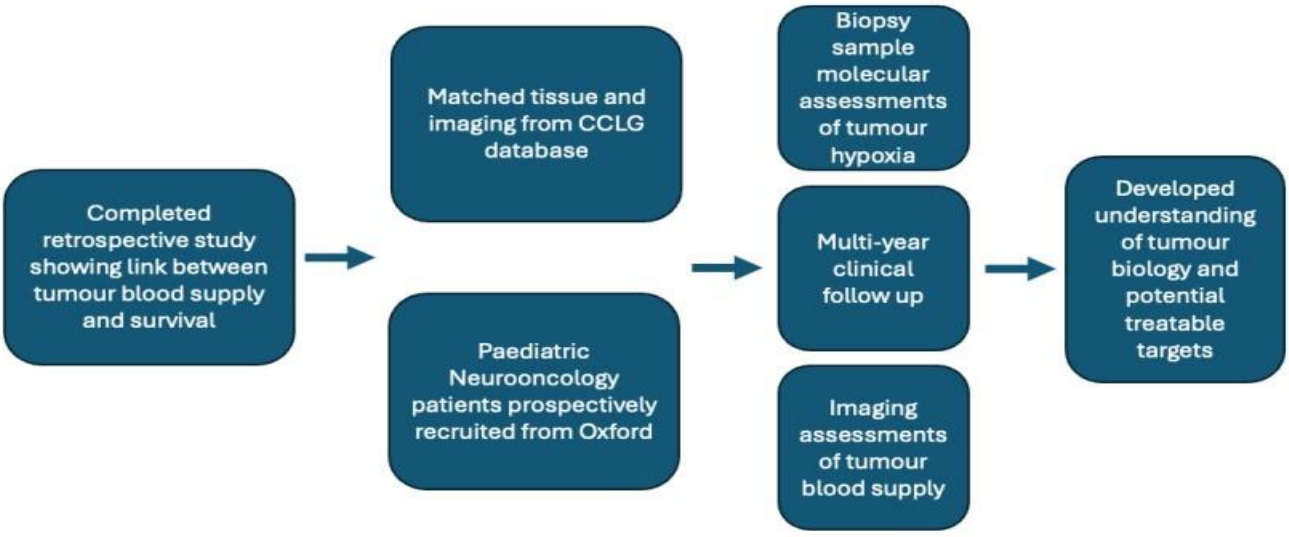

Figure 1 - overview of the DPhil project

The objectives of this DPhil project are:

1) **To assess the imaging-derived tumour blood supply and survival link in a prospective cohort study in Oxfordshire paediatric tumour patients.**

The student will drive a clinical study, in the case of a clinician DPhil they will assist in the consenting of children / parents with a newly diagnosed brain tumour after completing appropriate training and working in tandem with Dr Wilson, ensuring that data is collected from the MRI systems and biopsy samples from surgery. Further data will be collected from collaborating CCLG sites, with matched biopsy tissue from the CCLG tissue bank, to enrich the data set.
The student will, working with Dr James Grist, post-process the imaging data and analyse it using established methods, providing a measure of tumour blood supply to input into the pre-trained machine learning model to assign a 'high risk' or 'low risk' grouping. Participants will be followed throughout the lifetime of the DPhil to assess survival.
Outcome: This work will be publishable in leading journals such as Nature Communications, providing prospective validation of a machine learning model to identify children most at risk of death from a brain tumour.

2) **To derive the underlying biology linking imaging-derived measures of tumour blood supply, tumour vascularity, and hypoxia in biopsy derived samples.**

Whilst imaging data are collected, the student will collect tissue from surgical biopsy and assess it for markers of hypoxia, vascularity, and the underlying genetic regulation of hypoxic pathways, in the Hammond and Olcina labs. We have experience of this imaging/tissue analysis pipeline from the FIG trail that was conducted in Oxford in 2022-2023, assessing hypoxia in adult gliomas. Prospective high- and low-risk groupings derived from the machine-learning model will be used to help stratify data for statistical analysis to assess for differences in hypoxia and vascularity between groups.
Outcome: If successful, and hypoxia is a key difference within and between tumour types, then the student will have developed our understanding of tumour biology, identified potential treatable targets for future therapy development, and validated a potential biomarker for the effect of therapy. Data will be eminently publishable in high-impact journals such as Nature and the New England Journal of Medicine.

Translational potential of the project

This project is highly translational showing the direct link between retrospective clinical data, and a prospective clinical study with underlying biological mechanism elucidated. This is a lab-bench to bedside project with multiple future benefits:

1) A prospective validation of tumour blood supply as a prognostic marker for clinical use.
2) Identification of patients for next generation therapeutic studies
3) Development of our understanding of hypoxia biology and identification of potential treatable targets.

Training opportunities

A) **Scientific skill set development**

Dr Grist will provide the necessary in-depth training for the student to perform MRI imaging acquisition, reconstruction, image registration, post-processing, and analysis. Prof Hammond and Dr Olcina will provide the necessary training in lab-based molecular biology for biopsy sample analysis, as well as the associated quantification and analysis tools of the field. The student will become a multi-skilled professional with a wide-ranging scientific skill set.

B) **Clinical study management**

The student will be mentored by Dr Shaun Wilson, who will seek to develop their understanding of the clinical relevance of their work, as well as the day-to-day challenges of running clinical research studies in the NHS.
This will provide the student with the necessary skill set required to continue their career in clinical research.

Leadership and management

The student will be encouraged to engage with the University training courses in scientific leadership, research integrity, academic writing, and research grant writing. In turn, this will provide the student with the necessary skills to successfully undertake their DPhil and prepare them for the next stages of an academic career.

References

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2. Li, Y., Zhao, L. & Li, X. F. Hypoxia and the Tumor Microenvironment. *Technol Cancer Res Treat* **20**, 1–9 (2021).

23. Utilisation of MR linear accelerator technology for parallel development of lattice radiotherapy and oxygen-enhanced MR imaging – Geoff Higgins

Primary Supervisor: Geoff Higgins
Additional Supervisors: Kristoffer Petersson and Rob Rulach
Eligibility: Track 1 applicants are eligible to apply for this project

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 particularly for bulky, locally advanced tumours that usually do not respond well to conventional radiotherapy, without increasing the side-effects of treatment. Lattice radiotherapy is a type of spatially fractionated radiotherapy that 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 LRT 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 has increased the ability to accurately deliver radiotherapy to moving tumours and facilitate real time treatment adaptation. This proposal seeks to implement LRT 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 and proposed outcomes
The Department of 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 LRT. This project will seek to assess the capability of delivering LRT 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 LRT and will include analysis of the position, size and dose of high dose "spheres".

Subsequently, we will open a clinical study combining LRT 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 Rob Rulach) and medical physics (Kristoffer Petersson) and will involve detailed radiotherapy planning studies and clinical implementation.

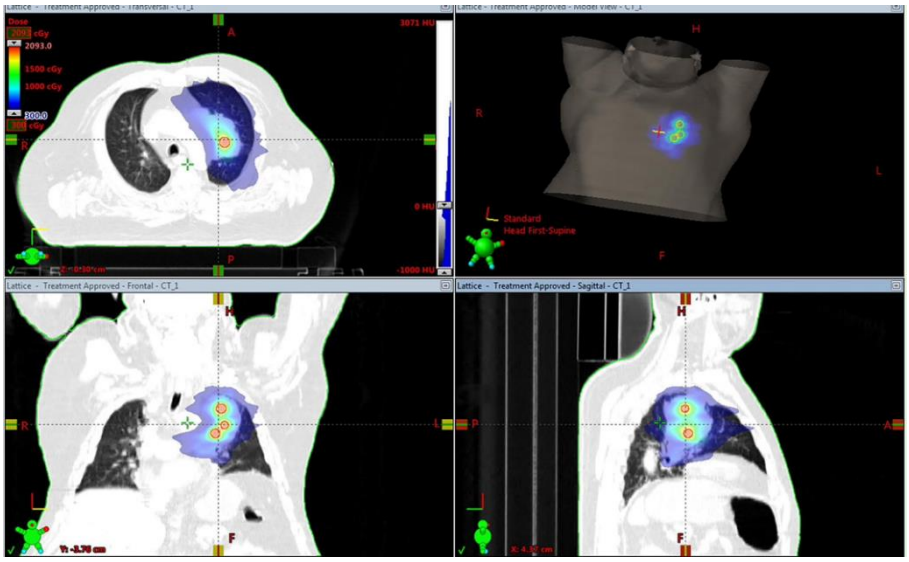

Fig. 1. Illustration of a SFRT treatment plan. Clockwise from top left; axial, model, coronal and sagittal views of colour wash dose distribution of 3 Gy around the tumour periphery and 18 Gy in the three 1.5 cm diameter dose peak spheres within the tumour. Adapted from (3).

Translational potential of the project

The development of MRL machines has advanced the capability to deliver radiotherapy in ways that have not previously been possible. LRT 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 LRT 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 be also be taught advanced radiotherapy planning methodology, functional MR image analysis and clinical trial experience.

Ideal student background: Suitable for a clinical oncology trainee, ideally having completed FRCR part 1 examinations.

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.

24. Multimodal adaptive risk prediction of diagnosis, prognosis and treatment response of prostate cancer using the QResearch database – Julia Hippisley-Cox

Primary Supervisor: Julia Hippisley-Cox
Additional Supervisors: Carol Coupland
Eligibility: Track 3 and Track 4 applicants are eligible to apply for this project

Abstract
Prostate cancer is the second most common cancer, with over 52000 men diagnosed annually, and the third most common cause of cancer death in the UK. Over 40% of prostate cancer is diagnosed at late stage, which is associated with poorer prognosis. The ten-year survival for early-stage prostate cancer is greater than 90% compared with 80% for Stage 3 and 19% for Stage 4 cancer at diagnosis[1]. Prostate cancer early diagnosis has increased significantly with the rise of PSA testing, but a major challenge is also overdiagnosis and overtreatment, resulting in invasive investigations and treatment for non-progressive prostate cancer that would not have caused cancer-related morbidity. The value of unsystematic PSA-based screening, which national guidelines currently recommend based on shared decision-making in primary care, is uncertain when considering benefits of reduced prostate cancer mortality weighed against medical harm from overdiagnosis [2,3]. While there have been advances in risk prediction models for early cancer detection in the last ten years, available tools e.g. the QCancer risk stratification system, generally only provide static risk estimates based on symptoms and patient characteristics at a point in time and do not include test or scan results [4]. Research indicates that trends over time in blood tests can provide predictive value for cancer beyond static results [5]. Risk scoring systems which are updated as further clinical information becomes available along the patient pathway are useful as they can inform further management steps. Promising early work also shows that machine learning methods applied to health record data can identify 'clinical phenotypes' of patients at risk of cancer [6]. Currently, prostate cancer treatment choice is based on histological grading, radiological staging and response to initial lines of hormone therapy in advanced tumours [7]. There are currently no adaptive interpretable decision tools supporting personalised management.

This project will develop and validate dynamic risk prediction tools to support early diagnosis and optimal personalised management of prostate cancer. This will be achieved by analysing routinely collected anonymised multimodal patient data from the QResearch database of GP electronic health records, which contains over 35 million patients and longitudinal data spanning 25 years. This is linked to NHS secondary care datasets - Hospital Episode Statistics, National Cancer Registration and Analysis Service, Systemic Anticancer Therapy (SACT) and Radiotherapy Dataset, imaging, and death registry data, which will be used to determine clinical outcomes of cancer. The QResearch dataset will be split into two subsets (1) of 24 million patients for model development, and (2) of 12 million patients for model validation. The CPRD GOLD dataset, an independent dataset, will be used for external model validation. Models will be developed using statistical methods, such as multinomial logistic regression, and compared to novel machine learning-derived models (supervised deep learning clustering models). We will then evaluate the performance of models applied to retrospective real patient cases, and usability of the risk prediction tools in practice.

Research objectives

1. Develop dynamic multimodal data-driven prediction models using the QResearch database that can determine the risk of men having aggressive prostate cancer based on most up-to-date clinical features, including test results.
    a. Compare performance of statistical models and machine learning models (convolutional neural networks).

   b. Compare performance of predictive models based on integration of primary care data and imaging data and those based on primary care data alone.
   c. Produce an 'optimised' risk prediction model for aggressive prostate cancer.

2. Develop dynamic prediction models to predict duration of survival for men who have prostate cancer, and which comprise updating estimates based on clinical features or events that arise over time.
3. Develop prediction models to predict response to radiotherapy, chemotherapy and hormone therapy and outcomes (benefits/risks) of treatments in men who have prostate cancer, using the QResearch database linked to systemic anti-cancer therapy and radiotherapy data sets.
4. Validation of derived models in external datasets.
5. Undertake clinical evaluation of risk prediction tools in practice.
6. Undertake qualitative study of the process of decision making regarding prostate cancer management for patients and their clinicians.

Proposed outcome: first-of-type adaptive decision-support tools to identify patients with high-risk prostate cancer and predict their prognosis. The risk scoring tools will update risk over time, incorporating investigation results and clinical information that become available as patients progress along clinical pathways.

This research will also deliver academic value for the field in the comparative evaluation of risk prediction pipelines encompassing different methodological approaches, e.g. statistical and machine learning, and multiple data types, the relative value of which for cancer risk modelling is not well understood. This project will support cross disciplinary collaborations between primary care, surgical sciences, and engineering, bring together experts in risk prediction modelling, big data, machine learning, oncology and qualitative research.

Translational potential

This project is one of the first attempts to unify multimodal health datasets from primary and secondary care to develop an adaptive risk tool for cancer detection and prognostication. It will develop first-of-type prediction models for cancer treatment response using a large primary care database linked to radiotherapy and chemotherapy data sets. This is enabled by unprecedentedly granular linked data available within the QResearch environment. The outputs of this work will enable a new approach to detection of high-risk prostate cancer, triaging for referral and investigations, and evidence-based personalised risk-based management, improving patient outcomes, reducing harm and enhancing resource use. To date, cancer decision support tools have not been systematically applied in practice in primary or specialist care, and unfamiliarity of patients, clinicians and service managers, who will need to know how to interpret and respond to results, is a challenge to translation. The qualitative study in this project will provide insight on how patients, clinicians, and systems currently approach decision-making, how they will respond to new tools and what is needed for successful implementation. The results of this project will inform further work to refine prediction tools, undertake software implementation and larger prospective evaluations of these systems.

Training opportunities

In addition to training in analytical methods mentioned above, the student will also be given training in systematic reviews, presentation of research findings, and preparation of grant applications. There will be the unique opportunity to be trained in use of QResearch (the largest primary care database in the UK linked to cancer, mortality and hospital records and the source for widely implemented risk prediction models) and qualitative methodology expertise of the Department of Primary Care Health Sciences. Additionally, there is collaboration opportunity with the Primary Care Clinical Trials Unit, Oxford-led NIHR Community Healthcare MedTech and In vitro Diagnostics Co-operative and multidisciplinary Oxford Centre for Early Cancer Detection, to support clinical evaluation and translation into practice of the innovative products.

Ideal student background: Successful applicants should ideally have a background in clinical research involving patients and big data research

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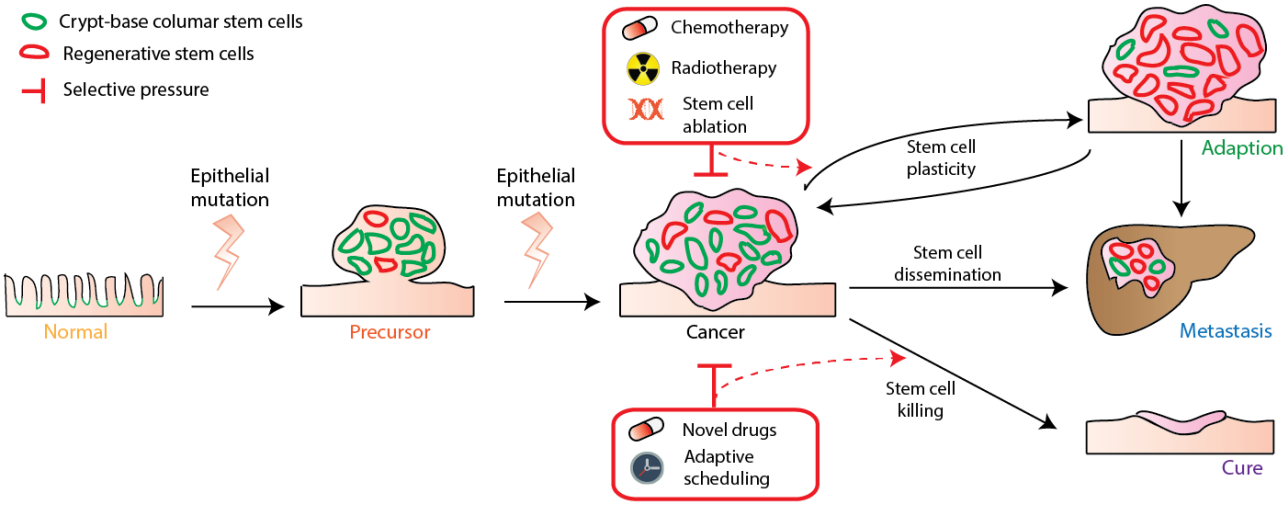
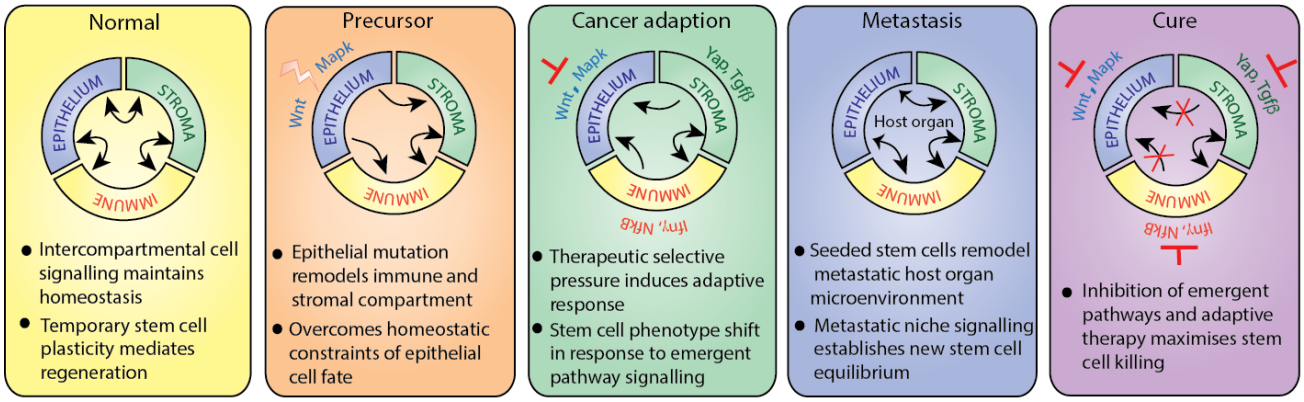
25. Manipulating the stem cell niche to prevent cancer cell adaptive plasticity – Simon Leedham

Primary Supervisor: Simon Leedham
Additional Supervisors: Helen Byrne
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract
Neoplasia is characterised by the activity of stem cells - from the impact of initiating (epi)mutation through to the emergence of therapy resistant clones and metastatic seeding. Stem cell activity is functionally supported by a niche, made up of surrounding matrix, stromal and immune cells. Cancer stem cells are capable of remodelling surrounding microenvironmental landscapes to generate ectopic stem cell niches where bidirectional crosstalk between cellular compartments maintains stem cell phenotype and promotes epithelial adaptive plasticity. Understanding the cell-extrinsic mechanisms driving cancer cell plasticity is key for tackling chemoresistance and developing efficacious new drugs. In this project we will use spatial biology with advanced mathematical analysis to map the cellular constituents of different cancer stem cell niches. We will then generate cancer xenograft models in niche constituent knockout mice to examine the effect of niche modulation on primary and secondary tumour engraftment, progression and capacity to adaptively respond to therapeutic selective pressures.





Background

Neoplasia is characterised by the activity of stem cells - from the impact of initiating (epi)mutation through to the emergence of therapy resistant clones and metastatic seeding. In mouse models, genetic inactivation of the key colorectal cancer driver gene, *Adenomatous Polyposis Coli* (*Apc*) in crypt base columnar cells (CBC's) precipitated rapid tumour induction (*Barker et al, Nature, 2009*). However, other studies subsequently showed that induction of inflammation or disruption of homeostatic morphogen gradients could also lead to neoplasia arising from alternative cell populations outside of the crypt base (termed regenerative stem cells). Our work has shown that colorectal cancers contain multiple populations of stem cells and that these cells are capable of shifting their phenotype (known as cellular plasticity), in response to therapeutic selective pressures, resulting in the rapid evolution of drug resistance (*Gilvasquez et al, Cell Stem Cell, 2022*). Understanding the mechanisms that underpin cellular plasticity and how to prevent it from happening following drug treatments, is key to improving drug response rates and improving patient cancer outcomes.

Research objectives

This project will explore the effect of the tumour microenvironment in mediating cancer stem cell plasticity and seek to mechanistically manipulate key cellular components to attenuate primary and metastatic tumour outgrowth. We will use human tissue and state-of-the art mouse models, deploy spatial biology techniques and analyse the resulting images using existing mathematical methods to assess cellular phenotypes within the tissue context.

Work package 1. Defining the cancer stem cell niche. Stem cell activity - defined by cell self-renewal and multipotency - is dependent on a stem cell niche, which is made up of surrounding stromal and immune cells and matricellular proteins. Cancer stem cells are capable of remodelling surrounding cellular landscapes to generate ectopic stem cell niches where bidirectional crosstalk between cellular compartments maintains stem cell phenotype and functionality, and promotes epithelial adaptive plasticity. Using improving spatial biology techniques (multiplex immunohistochemistry and spatial transcriptomics), capable of identifying epithelial, stromal, immune and matrix markers, we will map different cancer stem cell niches in human and mouse colorectal cancer and liver metastases to identify the cornerstone cell interactions.

Work package 2. Assessing the effect of genetic manipulation of niche cellular components on primary tumour engraftment and progression. Through the CRUK funded ACRCelerate project, we have access to advanced cancer mouse models and organoids across a range of complex mouse genotypes that are disease-positioned to model the human consensus molecular subtypes of CRC. By rectal endoscopic implantation of cancer organoids we can generate orthotopic xenograft mouse cancer models. WP1 will identify genotype-specific primary tumour niche stromal, immune and matrix components. WP2 will establish xenograft models in mice with genetic knockout of key niche components to examine the effect of niche manipulation on tumour engraftment, progression and capacity to respond to therapeutic selective pressures.

Work package 3. Assessing the effect of genetic manipulation of niche cellular components on liver metastasis. Circulating cancer stem cells must remodel the stromal and immune landscape of a distant metastatic host organ, in order to generate a supportive secondary tumour stem cell niche. Our own work has mapped these spatiotemporal events in the formation of liver metastases in advanced mouse models (*Canellas Socias et al, Nature 2022).* In this project, we will use a splenic injection model, allowing temporal control over metastatic cell seeding. We will examine the effect of key metastatic stem cell niche component knockout on secondary tumour engraftment and outgrowth potential, assess mouse genotype specific differences, and use multiplex imaging and maths analysis to look for stromal cell remodelling and innate immune cell infiltration. We will incorporate these data into ongoing development of agent-based models of metastatic outgrowth in a longstanding collaboration with Professor Byrne.

Translational potential

In colorectal cancer (CRC), standard-of-care combination therapies have not significantly advanced in decades, despite huge leaps forward in our understanding of CRC biology. Most treatments target the proliferating cancer epithelium alone, but treatment failure is frequently a consequence of dynamic and multicompartmental tumour adaption to therapy. In order to improve CRC cell targeting we need to understand the microenvironmental pathways that mediate adaptive cellular plasticity, and target them specifically to prevent the evolution of drug resistance. This project will use genetic models to manipulate key stem cell niche components in the hope of identifying new therapeutic targets for the next generation of drug development.

Training opportunities

This project will allow the student to develop their skills with wide and multidisciplinary scientific training. No prior experience is needed. The project will combine wet lab work using mouse models together with spatial biology and deep molecular phenotyping of mouse and human tissue. Students will have the opportunity to learn advanced mouse cancer techniques including endoscopic and splenic xenograft injection. Students will work with mathematical collaborators as the biological input to shared analysis of cellular relationships based on spatial biology dataset interrogation. Students are encouraged to develop their bioinformatic skills to enable them to analyse their own datasets and training for this will be provided. Clinical candidates in Gastroenterology can undertake training in GI family cancer clinics and endoscopy lists.

Return to Projects list

26. Combining polylipidoid and microneedles for safe and effective intradermal cancer vaccines – Carol Leung

Primary Supervisor: Carol Leung
Additional Supervisors: Molly Stevens and Tim Elliott
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract
Intradermal delivery of mRNA cancer vaccines is a well-known strategy for accessing resident antigen presenting cells to induce tumour-specific cytotoxic T-cell response.[1] Microneedles patches are an emerging alternative to intradermal injections. They offer enhanced patient compliance, consistent and simplified administration, and the potential for increased shelf-stability compared to standard aqueous vaccine formuhations.[2] However, their application is limited by the instability of lipid nanoparticles (LPNs), the clinically approved carrier for mRNA vaccines formulations. Moreover, the immune effects of LNP are incompletely understood, heading to an increased risk of adverse effects. Biodegradable polymers are an effective alternative delivery agent for RNA delivery. For example, poly (CBA- co-4-amino-1-butanol (ABOL)) (pABOL) polyplexes effectively encapsulate and deliver mRNA when injected intramuscularly, and display a short half-life and excellent biocompatibihity.[3] Additionally, lipid-like polymeric constructs (polylipidoids) have been developed which incorporate the advantages of both polymeric and lipid delivery systems. Polylipidoid formulations which readily transfect skin have not been demonstrated. In this project, we aim to develop polylipidoid formulations which effectively transfect dermal dendritic cells and combine them with microneedles intradermal delivery for more effective cancer vaccines.

Research objectives and proposed outcomes

1. **Development and screening of a library of polylipidoid nanoparticles.** Nanoparticles will be fabricated from a combinatorial library of polylipidoid formulations. Such nanoparticles will be characterised and screened for enhanced stability and transfection efficiency in dermal dendritic cells, compared to conventional LPNs formulations.
2. **Development of a microneedle patch for the delivery of polylipidoid-mRNA vaccines.** The best pohyhipidoid-mRNA nanoparticles candidates will be integrated into a microneedle patch for intradermal delivery, with the aim of improving both vaccine shelf-life and administration safety and efficacy.

Evaluation of immunogenicity and efficacy. The immunogenicity and efficacy of the developed vaccine system will be evaluated using both ex vivo human skin explant and appropriate animal models, aiming to demonstrate superior immune activation and cytotoxic T-cell responses compared to conventional LNP systems

Translational potential of the project
The translational potential of this project lies in its ability to significantly enhance cancer vaccine delivery, particularly for intradermal applications. By developing pohyhipidoid formulations that effectively transfect dermal dendritic cells, combined with microneedle patches for intradermal delivery, this project aims to address key limitations of current lipid nanoparticle (LNP)-based systems. This approach promises to improve vaccine stability, simplify administration, and enhance patient compliance. For patients, the benefits include a more effective immune response against cancer, reduced side effects due to better biocompatibility, and the convenience of self-administration through microneedles. Ultimately, this could lead to more accessible and effective cancer immunotherapies, improving patient outcomes and quality of life.

Training opportunities
Within the Stevens group the student will receive training in lipid, polymeric and polylipidoid nanoparticles synthesis and characterisation. Training will include but is not limited to dynamic light scattering (DLS), Single Particle Automated Raman Trapping Analysis (SPARTA), Ribogreen RNA assay for encapsulation efficiency, biocompatibility assays. The student will also receive training in microneedles fabrication and characterisation, including soft lithography and scanning electron microscopy (SEM). The student will also learn how to evaluate transfection efficiency both in vitro and in ex-vivo human skin explants. Within the Elliot's group the student will receive training in a wide variety of techniques including cell culture molecular biology, multiparameter flow cytometry and cellular immunology. In addition, the student will get Home Office Modular training to gain a Procedure Individual License for conducting animal research.

The proposed DPhil project ensures an enriching academic experience within a highly multidisciplinary, collaborative, and translational environment. The student will not only acquire technical proficiency in vaccine formulations, immunology and cancer biology, but also hone transferable skills typical of a doctorate programme, such as ethical research, data management, analysis and interpretation, science communication and many more. By working across disciplines and integrating diverse perspectives, the student will be primed to address complex issues effectively, making substantial contributions to both academia and real-world applications.

Ideal student background: While backgrounds in Pharmaceutical Sciences or Immunology are preferred, this project welcomes individuals from all disciplines who possess a strong passion and inclination for multidisciplinary and collaborative research.

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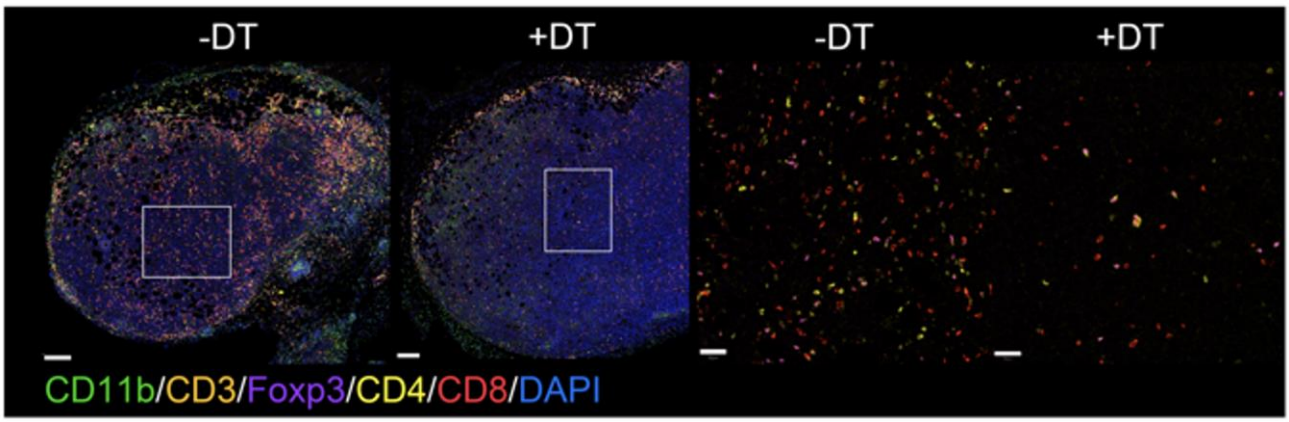
27. Understanding and exploiting cDC1-mediated cross-priming in cancer immunotherapy – Ignacio Melero

Primary Supervisor: Ignacio Melero
Additional Supervisors: Maria Aggleakopoulou
Eligibility: Track 2 applicants are eligible to apply for this project

Abstract
Cancer immunotherapy using immune checkpoint inhibitors (ICI) has revolutionised oncology practice, but fails to show clinical benefit in 70-90% of cancer patients, is not successful for some cancer types, and can have severe side effects[1]. Professional antigen presenting cells (APCs), including dendritic cells (DCs) play an instrumental role in eliciting anti-tumour cytotoxic T lymphocyte (CTL) responses. DCs orchestrate the differentiation and expansion of anti-tumour $CD8^+$ T cell responses via the presentation of tumour-derived antigens to naïve $CD8^+$ T cells, a process called cross-presentation/cross-priming, as well as via the provision of functional cues. Specialised 'conventional type-1 dendritic cells' (cDC1s) have a superior antigen cross-presentation ability to $CD8^+$ T cells and are critical for all known cancer immunotherapies to be effective[2]. Studies by us and others, using cDC1-deficient mice and XCR1-DTR transgenic mice have demonstrated the necessity of cDC1s in effective cancer immunotherapy[3,4,5]. In addition, the presence of cDC1s in the tumour microenvironment (TME) has been positively correlated with the abundance of tumour-infiltrating T cells[6]. We hypothesize that cDC1s offer underexploited opportunities to improve the efficacy and to define biomarkers of effective cancer immunotherapy, therefore the elucidation of the mechanisms regulating their superior antigen cross-presentation ability and function, is of high priority. To explore this, we will use mouse models to determine which tumour neoantigens are cross-presented by cDC1s using advanced proteomics and also mechanistically study the immunological synapse formation between cDC1 and $CD8^+$ T cells.

Figure 1. Depletion of cDC1s in XCR1-DTR mice with diphtheria toxin causes a dramatic reduction of the T cell infiltrate in MC38 engrafted tumours as assessed with a panel of multiplex tissue immunofluorescence

Low magnification High magnification



Research objectives
First, we will apply mass spectrometry-based immunopeptidome profiling approaches[7], established in the Adamopoulou group, to explore the repertoire of MHC-I-bound peptides presented by cDC1 cells within murine tumours and in tumour draining lymph nodes (DLNs). Using already refined immunopeptidome profiling

approaches for small cell numbers isolated from small tissue specimens[7], as well as for isolated and *in vitro* expanded mouse cDC1 cells established in the Melero group, we will profile tumour epitopes that are cross-presented by cDC1 cells. The comparison of the immunopeptidomes derived by murine tumours and tumour DLNs will shed light on the relevance of the location of cDC1s for adequate $CD8^+$ T cell priming. We will use the Colon Tumour 26 (CT26) transfer model, that is a colorectal carcinoma model, where around 85% of the total $CD8^+$ T cell response to 3 epitopes encoded by gp70 have been mapped, or the MC38 colorectal carcinoma transfer model where MC38 cells will be transfected with different sequences of surrogate antigens (i.e. ovalbumin) to interrogate rules of antigen cross-presentation by cDC1s[8]. Novel sFLT-3-based *in vivo* treatments will be applied to augment the numbers of cDC1s in tumour tissues and facilitate the analysis.

Secondly, we will use advanced microscopy and imaging approaches, mass spectrometry and multicolour flow cytometry to characterise the immunological synapse (IS) formation between cDC1s and $CD8^+$ T cells, in collaboration with the Dustin group[9]. These studies will elucidate the help cDC1s provide to $CD8^+$ T cells and the TCR-microvesicles released. This will lead to the identification of receptor-ligand interactions that either enhance or repress the priming or expansion of antigen-specific $CD8^+$ T cell responses following cDC1:$CD8^+$ T cell immunological synapse formation. The role of specific interactions of receptor-ligand pairs, such as CD28-CD80, CD70-CD27, NKG2A-H-2Qa CD137-CD137L, LFA-1/ICAM-1, CD8/MHC-I, IL15-IL15Rα and PD-1/PD-L1 and of paracrine cytokines at immunological synapses of cDC1 and $CD8^+$ T cells are yet not clear and we hypothesize that they can be critical for the outcome of $CD8^+$ T cell cross-priming or expansion. cDC1s are the only physiological source of IL-12[10], among various other costimulatory signals, necessary for the activation of CTLs. These studies will determine whether induction of IL-12 and of costimulatory molecules, such as CD70 or CD137 are sufficient for the activation and/or expansion of CTLs. Furthermore, investigation of the differences of $CD8^+$ T cell priming by intratumour- or tumour DLN-derived cDC1s will offer insight of the interplay of cDC1 cells migrating from the TME to tumour DLNs and the resident cDC1 cells in the tumour, in effective cancer immunotherapy.

Translational potential

These studies aim to shed light on the immunobiology of cDC1 cells and offer new mechanistic insights that could be exploited therapeutically, in combination with cancer immunotherapy. Importantly, data obtained from these studies can set the foundation for clinical trials that investigate cross-priming-based immunotherapies in patients with cancer. Furthermore, the profiling of tumour antigens that are cross-presented by cDC1 cells will improve immunisation strategies that enhance T-cell infiltration into tumours and the monitoring of immune responses upon cancer immunotherapy treatments.

Training opportunities

The DPhil student will be based at the Old Road Campus Research Building. This project provides broad training in cancer immunology, covering a range of cellular and functional immune assays. The student will have access to cutting-edge technologies such as mouse tumour models, cDC1/CD8 co-cultures systems, advanced microscopy, multicolour flow and/or spectral cytometry, mass spectrometry-based immunopeptidomics and comparative bioinformatics on proteomic sequences. The project will enhance their skills in experimental design, data interpretation, literature review, and scientific writing. The student will also have multiple opportunities to present their findings at inter-departmental seminar series and national and international conferences

Ideal student background : You should hold a degree in a relevant discipline such as biology or immunology or related field. A Master's degree in Immunology/ Cancer Immunology will be an advantage but is not a prerequisite. Previous laboratory experience in cellular immunology (e.g. cell culture, flow cytometry, and/or microscopy) will be an advantage but is not required, as the DPhil student will be trained and will have daily supervision as required by senior lab members and collaborators. Excellent communication skills and ability to work as part of a team are essential.

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28. How does mechanical force dictate tumor initiation, progression and treatment response? – Kim Midwood

Primary Supervisor: Kim Midwood
Additional Supervisors: Caroline Morell and Adrien Hallou
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract
All tissues are made up of cells surrounded and supported by extracellular matrix. Matrix deposition in healthy tissues is a major risk factor for cancer; more fibrous tissues are up to 125 times more likely to develop tumors [1], resulting from altered biochemical and biomechanical cues that constrain or permit tumor initiation and field cancerization [2]. Fibrosis within the tumor microenvironment (TME) is also associated with poor prognosis and treatment response; dense and stiff matrix prevents drug access, causes immune exclusion and reprogrammed cell behaviour to facilitate tumor growth and metastasis [3]. Despite this, current therapeutic strategies to reduce tumor fibrosis by ablating or inhibiting cancer associated fibroblasts (CAFs), or by proteolytic digestion of the matrix have not met success in clinical trials. One major obstacle to unlocking tumor matrix is that the non-cellular component of the TME, which can constitute up to 80% of tumor volume in very desmoplastic disease, is extraordinarily complex, and plays both pro- and anti-tumorigenic roles [4]. Targeting single pathogenic matrix molecules is effective in pre-clinical validation [5]. However, it is not known how integrated signals from fibrotic tissue niches mediate locally adapted cell behaviour to drive disease. We generated transcriptomic blueprints that code for immune-infiltrated or immune-excluded tumors, that respond well or poorly to immunotherapy respectively. Mapping this list of ingredients back to tumor tissue by high-plex imaging revealed the underlying architecture of differential treatment response, identifying unexpected cross talk between matrix and tumor resident CAFs, immune and epithelial cells (Figure 1). Here, we will integrate spatial transcriptomic profiling with analysis of local mechanical forces and tissue mechanical properties (e.g. elasticity, viscoelasticity), to the assess the contribution of changes in tissue biophysics to treatment resistance, leveraging this information to design better therapies for hard to treat cancers.

Research objectives and proposed outcomes:
Aim 1. Map mechanical force at scale in tumor tissues: We will measure mechanical forces using image-based force inference and mechanical properties using in situ atomic force microscopy (AFM) or nanoindentation on tissue sections across unaffected, tumor adjacent and tumor tissue from immune-infiltrated or -excluded head and neck cancer. Data will be integrated with spatial gene expression data to simultaneously annotate the transcriptional, morphological and mechanical state of cells at single-cell resolution [6]. This aim will provide insight into mechanisms that promote boundary formation during tumor development, and the role of mechano-responsive regulatory pathways driving cell organization/spatial patterning in each tumor type.

Aim 2. Validate mechanosensitive signalling associated with treatment resistance: Gene modules whose expression patterns are significantly associated with the mechanical state of each tumor type will be further examined by imaging protein expression/localization in healthy tissue, and treatment resistant or responsive tumors. The role of genes of interest will be assessed by CRISPR mediated knock-in/out using co-culture model systems comprising CAFs, tumor and immune cells in 3D microenvironments of tailored mechanical properties.

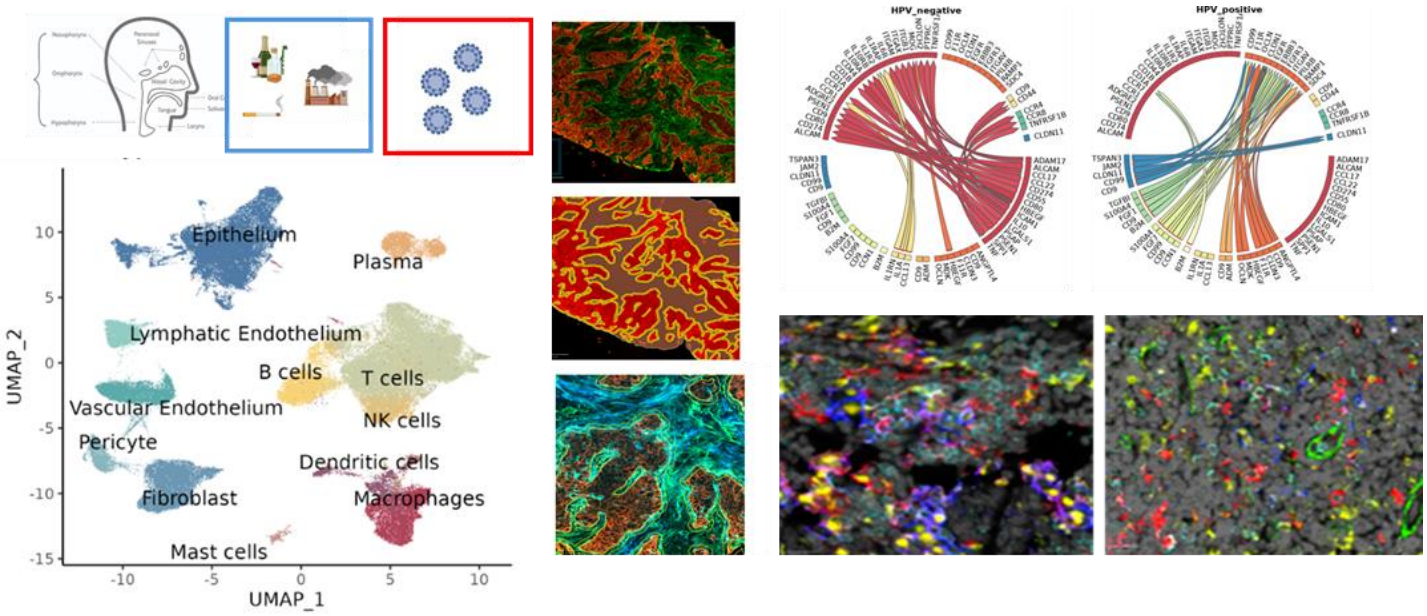

Figure 1. People with head and neck cancer at the same anatomical site, but of different aetiology and treatment response, have distinct matrix patterns that dictate tumor and stromal architecture, and immune infiltration and treatment response.

Translational potential & relevance to cancer research and patient care

We will focus this project on head and neck squamous cell carcinoma (HNSCC) with the view to applying our new methods to other solid tumors to interrogate tissue specific and conserved pathogenic mechanisms. HNSCC is the 6th most common cancer in the world. In the last 20 years, cases have increased by 34% in the UK, with a steeper increase in women than men. Rates are projected to continue increasing by 3% per year. Survival from HNSCC varies based on subsite, stage and diagnosis and cause, but is generally poor with a 5-year survival is as low as 40% for some patients. Suicide rates in survivors are 64.3 per 100,000 cases, second only to pancreatic cancer, reflecting the severity of the impact of HNSCC on quality of life and mental health. Despite improvements in treatment (targeted radiotherapy, immunotherapy), there has been no consistent translation to improved outcomes for people with HNSCC, and in recent clinical trials only a small minority of patients saw benefit from immunotherapy. There is therefore an urgent unmet need to better understand this disease to find ways to predict who will benefit from existing therapies and to develop new treatment modalities for those that will not.

Training opportunities

The successful applicant will benefit from a multidisciplinary supervisory team with access to cutting edge platforms including PIUMA nanoindentation, AFM and high-resolution tissue and cell imaging, as well as training in integration of spatial transcriptomic data sets with force measurement using coding in python, R and mathematical modelling, mechanistic validation of emerging signalling pathways using CAF-tumor-immune cell co-culture models and cellular immunology techniques.

Ideal student background: A strong interest in tumor immunology, tissue biology and mechanobiology, with hands on experience in wet or dry lab research, will be beneficial for this project.

References:

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Return to Projects list

29. Integrating multiparametric MRI with spatial transcriptomics to identify clinically relevant "Radio-Spatial Genomic" features of prostate cancer using artificial intelligence – Ian Mills

Primary Supervisor: Ian Mills
Additional Supervisors: Richard Bryant
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Prostate cancer (PCa) is the most common malignancy amongst men in the United Kingdom [1]. One of the key challenges in the clinical management of PCa involves risk-stratification, to precisely identify the subgroup of men at highest risk of progressing from localised to metastatic disease, and who therefore warrant radical treatment, whilst avoiding overtreatment and associated side-effects for those with lower risk disease who do not need treatment. Multiparametric MRI (mpMRI) has become a cornerstone in the PCa diagnostic pathway, identifying individuals who require a prostate biopsy, and improving the diagnostic biopsy sampling process, whilst reducing unnecessary oversampling. Following pre-biopsy MRI, a prostate biopsy remains an essential next step in the diagnostic pathway to ensure accurate detection of clinically important disease [2], given issues such as moderate inter-reader reliability, false-positive inflammatory lesions and MRI-invisible cancer.

Spatial genomic profiling has emerged as a powerful technique that provides valuable information with which to characterise PCa heterogeneity, and evaluate clonal dynamics, to identify features of potentially aggressive or lethal disease [3,4]. Spatial genomic profiling is currently prohibitively expensive for routine use in clinical practice. However, there is an exciting opportunity to potentially identify important biological features of PCa (from the spatial transcriptomics) within the mpMRI imaging (by amalgamating, or integrating, the clinical parameters, mpMRI radiomics and spatial transcriptomics, into so-called "Radio-spatial genomics"). This novel "Radio-Spatial Genomics" platform has to date not been undertaken for PCa, and therefore offers an exciting opportunity to identify mpMRI radiomic features associated with important biological aspects of PCa linked to an aggressive disease phenotype based on parallel investigation of spatially resolved transcriptomics from corresponding tissue samples. As pre-biopsy mpMRI is now routinely used in the diagnostic pathway for PCa, leveraging the potential wealth of information provided by mpMRI radiomics, much of which is not routinely used in image reporting (such as quantitative features, including textural, grayscale and shape features), may offer additional information beyond that currently available to better risk-stratify patients.

The research team in Oxford has expertise in high-throughput spatial transcriptomics biology, with this technique having been performed in samples from 10 patients with PCa who underwent radical prostatectomy (surgical removal of the prostate), and who have pre-operative mpMRI imaging for analysis. The DPhil student will work within the Nuffield Department of Surgical Sciences, in collaboration with the medical image analysis group in the Big Data Institute, and in collaboration with Urology and Radiology colleagues, to develop and refine a mpMRI and spatial transcriptomics co-registration workflow, to include the use of artificial intelligence (AI)/deep learning. This will involve establishing a novel pipeline to infer the biopsy of "clinically significant"/high risk PCa from the mpMRI images, using an integrated analysis of the spatial transcriptomics, using an integrated analysis approach of paired mpMRI and spatial biology datasets from these highly annotated and well-curated cases. In the longer term, this "Radio- Spatial Genomics" technique may be utilised to infer important biological features associated with an aggressive clinically relevant PCa phenotype from the mpMRI radiology imaging, to improve risk stratification by incorporating

novel radiological features from the diagnostic mpMRI imaging.

Research objectives

- To develop and validate mpMRI and spatial transcriptomics histology co-registration. To achieve this first objective, a convolutional neural network for co-registration will be evaluated using an existing resource of multi-section spatial transcriptomics and pre-operative mpMRI available from a small well-annotated patient cohort. **Collaborators**: Dr MacPherson (Consultant Radiologist), Dr Grist (MRI physicist), Associate Professor Papiez (Medical Image Analysis expert), Dr Colling (Consultant Histopathologist), Dr Figiel (Postdoctoral Scientist), Prof Mills (PCa Researcher), Prof Woodcock (Data Scientist), Prof Lamb (Ca Researcher and Urologist), Prof Bryant (PCa Researcher and Urologist).
- To identify mpMRI radiomic features that correlate with biologically important transcriptomic signatures from spatial transcriptomic analyses, potentially associated with adverse clinical outcomes. Having generated an integrated "Radio-Spatial Genomics" resource in Objective 1), this model will be used to infer important biological features of PCa from the Spatial Transcriptomic data to the Radiological data. This approach will be used to identify radiomic features from genomic features, with these being a potential surrogate for clinical outcomes. **Collaborators**: Dr MacPherson (Consultant Radiologist), Dr Grist (MRI physicist), Associate Professor Papiez (Medical Image Analysis expert), Dr Colling (Consultant Histopathologist), Dr Figiel (Postdoctoral Scientist), Prof Mills (PCa Researcher), Prof Woodcock (Data Scientist), Prof Lamb (PCa Researcher and Urologist), and Prof Bryant (PCa Researcher and Urologist).

Translational potential

This project may lead to evaluation of radiomic features in a cohort of men with pre-operative mpMRI and longer-term follow-up data to correlate inferred "Radio-Spatial Genomics" with phenotype. These features may be evaluated as an adjunct to improve non-invasive risk-stratification for patients with newly diagnosed PCa.

Training opportunities

The student will be provided with medical image analysis training and application of a convolutional neural network during attachment at the Big Data Institute under the supervision of Prof Papiez. Training will be provided by Dr Grist on mpMRI physics and quantitative feature extraction. Dr MacPherson will provide training in clinical image analysis and interpretation, and Dr Colling will provide training in histology and will work with Prof Mills, Prof Woodcock and Dr Figiel to evaluate transcriptomic signatures. Prof Lamb and Prof Bryant will provide guidance on clinical translation and overall direction of the research project.

Ideal student background: Successful applicants should ideally have a background in translational and clinical research, involving patient data

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30. Multi-cancer early detection testing in clinical practice – Brian Nicholson

Primary Supervisor: Brian Nicholson
Additional Supervisors: Eva Morris
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

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 and proposed outcomes.
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 of the project
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 Health Research Centre works upstream and downstream of the CE-marking process to both influence the development of novel technologies and the evaluation of clinic-ready products.

Ideal student background: The necessary skillset of the student will be determined by the area of study. For example, a clinical trainee or health services researcher could contribute to clinical implementation and pathway development, a non-clinical scientist to biobanking and analysis of samples, and an aspiring statistician or epidemiologist could develop the models to select patients for MCED testing.

31. Epigenetic control of cancer cell phenotypes via nuclear F-actin based chromosome motility – Eric O'Neill

Primary Supervisor: Eric O'Neill
Additional Supervisors: Yang Shi
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

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 differentiation[6]. 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 the mechanical environment and extra cellular matrix influences nuclear actin filaments to influence cell phenotype. We will explore how nuclear actin influences the stabilisation of cell phenotypes through mobilising chromatin and phase separated boundaries[7] and impacts tumour progression from benign lesions in pancreatic cancer.

Research objectives and proposed outcomes
(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 of the project.
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[8]. 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[9], 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[10], 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.

Ideal student background: Capability in cell and molecular biology techniques is preferential but not essential. Prior experience in microscopy would be an advantage but training can be provided.

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32. Assessment of Oesophageal cancer patient responses to immunotherapy via human tissue avatars – Richard Owen

Primary Supervisor: Richard Owen
Additional Supervisors: Eric O'Neill
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract
Currently, the majority of in vitro therapeutic testing is carried out using patient-derived cell lines, xenografts (PDX) and genetically engineered mouse models (GEMMs). While murine models provide valuable information about the systemic effects of therapeutics, GEMMs fail to capture the genomic heterogeneity of native tumours and PDX models are challenging to establish orthotopically and do not allow for the interaction of tumour with a functional immune system to be studied (Kim et al., 2009). Introduction of patient- derived organoids has allowed in vitro analysis of treatment interactions with three-dimensional tumour structures (Ooft et al., 2019) but, similarly to xenograft models, frequently fails to capture the complexity of the TME (Larsen et al., 2021). Recent efforts move in the direction of incorporating multiple different cell types and vascular mimics to reconstruct tumour complexities (Neal et al., 2018). Patient-derived tumour slices provide a platform through which tumour, stroma and immune infiltrate can be studied in their native architecture (Ghaderi et al., 2020; Kokkinos et al., 2021). Through this system therapeutics can be investigated for their impact throughout the tumour, allowing analysis of intra-patient variation in a clinically relevant timeframe.
O'Neill lab has developed the use of live patient-derived tumour slices for dissection of pancreatic cancer microenvironment and investigation of therapy responses. Methods have established to maintain superior cellular fitness and preservation of tumour microenvironment compared to standard cultures, organoids or spheroids. Analysis of transcriptomic changes induced by a combination of therapies aimed to target metabolic reprogramming treatment shows the potential of the platform to interrogate treatment responses across all cellular compartments of the microenvironment, in particular immune, in an unprecedented manner. Having demonstrated that organotypic tumour slices can maintain viability and provide novel insights enhancing both novel therapeutic discovery and precision medicine to improve current standard of care.

Oesophageal cancer is the sixth leading cause of cancer mortality worldwide (Bray et al., 2018). The predominant subtype in the western world, oesophageal adenocarcinoma, is among the cancer types with the highest increase in incidence over the past few decades (Devesa et al., 1998; Fitzgerald, 2004; Groulx et al., 2020; Lepage et al., 2008; Pennathur et al., 2013). About 40% of oesophageal cancers present with distant metastases at diagnosis (Smyth et al., 2017) and for these inoperable patients, median overall survival (OS) with conventional agents is less than one year (Cunningham et al., 2008; Dijksterhuis et al., 2019; Janmaat et al., 2017; Jatoi et al., 2006; Waddell et al., 2013). Treatment regimens using αPD-1 with chemotherapy have been approved and an Oxford-based trial (LUD2015-005) recently performed comprehensive clinical and molecular profiling throughout treatment using a combination of whole genome sequencing (WGS), single-cell RNA-sequencing (scRNA- seq), and bulk RNA-sequencing (bulk RNA-seq) to identify patients that benefit. Treatment-responsive molecular signatures were identified that effectively predict response and resistance to first line αPD-1 and also predicted long-term αPD-1 outcomes in other settings (Carroll et al. 2023). Notably, high PD-L1 expression and tumour mutational burden composed indicators to establish pre-treatment biomarkers that could improve prediction of long-term outcomes of αPD-1 treatment.

Research outline

This project is aimed to develop live tissue patient avatars from oesophageal adenocarcinoma biopsies using the technology validated for immune monitoring of pancreatic cancer avatars in the O'Neill lab. We aim to engineer this approach to screen for patients susceptible to αPD-1 therapy and a platform to assess further immune-therapies as potential combinations for patients not served by the pre-treatment biomarkers I have found (Carroll et al. 2023).

33. Characterising the tumour microenvironment of oesophageal adenocarcinoma and impact of mechanical properties – Eileen Parkes

Primary Supervisor: Eileen Parkes
Additional Supervisors: Adrien Hallou
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract
With the advent of immune checkpoint blockade as standard of care in many cancers, such as oesophageal adenocarcinoma (OAC), there is now a pressing need to identify new combination strategies for the very high proportion of immune checkpoint blockade-resistant or non-responding diseases. OAC is a solid cancer of unmet need, on course to impact increasing numbers of people and yet poorly characterised in terms of tumour microenvironment. OAC is a disease of high chromosomal instability, a critical feature that has downstream impacts on immune responses. Work in the Parkes lab has identified a specific inflammation phenotype in chromosomally unstable, cGAS-STING pathway active cancers. Using single cell data and digital pathology approaches we previously characterised the myeloid infiltrate in chromosomally unstable cancers. CIN-high OAC has high expression of genes involved in organisation and remodelling of the extracellular matrix, which determines the structure of the tumour and induces a distinct fibroblast phenotype within the microenvironment. How this in turn affects the immune compartment of CIN-high OAC cancers remains to be characterised. In addition, the link between CIN-status, tumour microenvironment and response to immunotherapy has still to be explored. In this project, the candidate will characterise the extracellular matrix and mechanical properties of the tumour and its microenvironment in chromosomal instability that subsequently impact the immune response.

Research objectives and proposed outcomes
Here we will investigate the tumour microenviroment of CIN-high and CIN-low OAC using fresh tissue biopsies and blood collected from OAC patients alongside *in vitro* modelling using a range of established 2D and 3D models. The aims are:

(1) *Profile the fibroblast and extracellular matrix components in the tumour microenvironment of CIN-high and CIN-low OAC*. Using multiparameter flow cytometry, fresh and untreated OAC tissue and blood samples will be processed and analysed, to phenotype the tumour microenvironment, both in terms of immune cells and fibroblasts. To complement this, a functional assessment of the myeloid compartment within the microenvironment will be performed, in order to determine the pro-tumour or anti-tumour role of such a plastic subset of cells (i.e. their ability to suppress T cell proliferation). We will also use multiplex digital pathology approaches to identify relationships between chromosomal instability and extracellular matrix deposition (figure 1). Collaborative partners in this aim include OUH surgeons and gastroenterologists, as well as the tissue banking team. **Outcome:** Characterisation of the tumour microenvironment in OAC and identification of distinctive matrix and fibroblasts phenotypes in CIN-high vs. CIN-low disease.

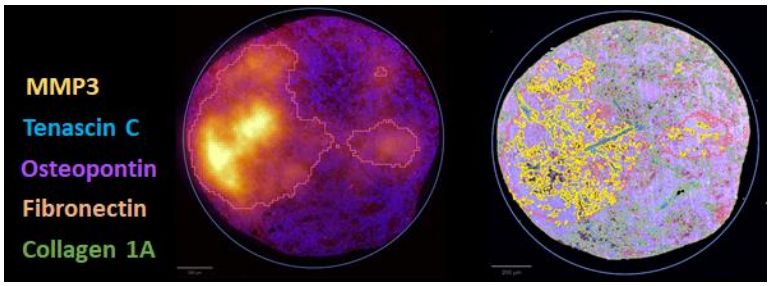

Figure 1: Multiplex immunofluorescence characterising matrix deposition in regions of high chromosomal instability

(2) *OAC tumour microenvironment 3D modelling*. Organoids and matched fibroblasts were previously established from patient samples (Parkes lab). Building on this, we aim to obtain 3D co-cultures to include the myeloid compartment previously characterised (in aim 1) and represent a more accurate OAC tumour microenvironment. This will help dissect the complex crosstalk between the tumour cells, the fibroblasts and the immune cells. Healthy monocytes and granulocytes will be added to organoid + fibroblast cultures and the following questions will be asked: is the CIN-type and fibroblast phenotype inducing polarisation of the myeloid cells to become tumour-promoting, i.e. are these cells immunosuppressive after co-culture? What mechanisms underlie this polarisation event (e.g. cytokines secretion, contact dependent, mechanical environment)? **Outcome**: Novel organoid-based co-culture assays to characterise the interaction between myeloid cells, fibroblasts and OAC tumour cells.

(3) *Interrogate the impact of CIN-high on tumour mechanical properties.* Using an innovative digital pathology platform established in the Hallou Lab, which permit profiling at single-cell resolution the mechanical and transcriptional properties of cells in a tissue section, we will characterise the mechanical forces across CIN-high and CIN-low tumours and the mechanical properties of the associated extracellular matrix, using existing and novel samples. Multiplex panels characterising YAP-TAZ signalling and other mechanotransduction effectors and pathways will be optimised and applied. We will complement this with spatial transcriptomics. Functionally, the candidate will investigate the impact of tissue stiffness and matrix deposition on immune cell behaviour and activity using PBMCs. The Hallou Lab is a leader in interrogation of cell and tissue mechanical properties and the student will develop novel experimental methodology and algorithms alongside the team in this project. In this way, we will investigate the relationship between CIN and tumour mechanical properties including both active forces generated by cells and mechanical properties such as cells and extracellular matrix stiffness.

Translational potential of the project

This proposal will identify and characterise new therapeutic opportunities in the immuno-oncology space by dissecting the interactions between chromosomal instability and mechanical properties of cancer, which are associated with therapeutic resistance. Interrogating the pathways that are activated by CIN that contribute to or are influenced by tissue mechanical properties will identify novel targets and the future development of combination immuno-oncology strategies. Moreover, the characterisation of the microenvironment of CIN-high OAC will inform on approaches potentially relevant to other CIN-high solid cancers. Translational potential is ensured by Dr Lizzy Smyth, a global leader in translation and implementation of clincial trials in OAC.

Training opportunities

The successful applicant will be co-supervised by Professor Eileen Parkes, Dr Adrien Hallou and Dr Lizzy Smyty. The student/clinical researcher will be given training in immunology and molecular biology techniques including flow cytometry, 3D culturing of organoid models, primary tissue processing, cell sorting, cell and tissue mechanics, digital pathology analysis as well as bioinformatic training for single cell and spatial RNAseq analysis. The Parkes and Hallou labs are established teams including postdoctoral fellows, DPhil students as well as technical support. Attendance at clinics and discussion regarding clinical impact will led by Dr Smyth and her team. Students will also have the opportunity to attend bioinformatics training to learn or advance their bioinformatic skills. In addition to the scientific aspect of the research project, the student/clinical researcher will benefit enormously from the career development programmes at the Oncology and Kennedy Institutes.

Ideal student background: Students having obtained or in the process of obtaining a medical degree would be preferred for this application. Ability to integrate data from various sources and basic knowledge of R would be desirable.

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Hallou, He, Simons, Dumitrascu. A computational pipeline for spatial mechano-transcriptomics. In press, Nature Methods (2024) BioRxiv 2023.08.03.551894.

34. Exploring the role of Oncostatin M in the stromal-epithelial cross talk during inflammatory bowel disease and tumorigenesis – Fiona Powrie

Primary Supervisor: Fiona Powrie
Additional Supervisors: Mathilde Pohin
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract
IBD is a chronic inflammatory disease of the gastrointestinal tract characterised by a breakdown of the epithelial barrier leading to ulceration and tissue injury. Patients with IBD have a higher risk of CRC, which increases with the duration, extent, and degree of inflammation. Upon tissue injury, epithelial cell repair requires the activation of a "wound-associated epithelial" (WAE) gene program induced by signals from the lamina propria amongst which stromal cells exert a critical role [1]. In IBD tissue with ulceration (wound that does not heal), we have shown that fibroblasts are actively remodelled by the inflammatory landscape and in particular by neutrophil derived cytokines such as Oncostatin M (OSM) [2,3]. Interestingly, this inflammatory fibroblast (FAP+ PDPN+) program is also found in CRC patients alongside neutrophils infiltrates (MPO) suggesting that mechanisms of wound healing in IBD and in CRC are overlapping (Figure 1A). The concept of molecular similarities between tumors and wounds and the description of carcinogenesis as " a wound that never heals" has been in the literature for decades [4] and the WAE gene program is enriched in CRC reflecting the presence of stromal signals promoting epithelium repair [5]. OSM receptor (OSMR) is specifically expressed by stromal cells in the intestine and we have previously shown that targeting OSM signalling in the Helicobacter hepaticus (Hh) + antIL10R mouse colitis model featuring hyperproliferation of the epithelium is beneficial (West et al). Moreover, our preliminary work shows that *OSM* expression is higher in tumour than in the inflamed adjacent tissue of a colitis associated cancer mouse model (Hh+ Azoxymethane AOm) (Figure 1B). We hypothesise

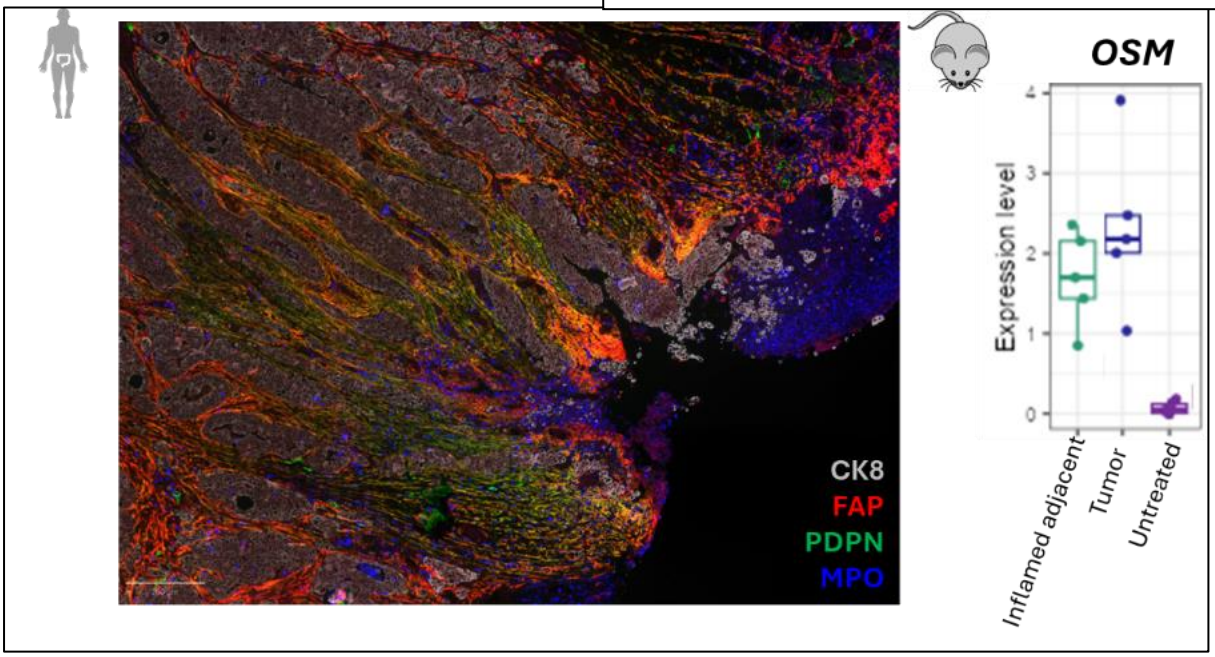

Figure 1. A. Immunohistostaining of resected human CRC tissue showing the presence of neutrophils (Myeloperoxydase MPO) and inflammatory fibroblasts (Podoplanin PDPN, Fibroblast activated Protein FAP) in the tumour (Cytokeratin CK8). B. Transcriptomic expression of *OSM* in the inflamed tissue, tumour distal colon of a HhAOM colitis associated cancer mouse model.

that OSM is required for healing of the epithelium which is impaired in IBD but overactive in CRC. As such, OSM function may be detrimental in CRC. Using our mouse models of colitis, wound healing and cancer, we will characterise the role of OSM in the stromal – epithelium cross talk to understand its contribution to IBD and CRC pathology.

Research objectives and proposed outcomes

Aim 1. Determining the function of Oncostatin M in intestinal wound healing and in colitis. We will use an intestinal biopsy punch would healing model and a microbe-driven colitis mouse models to determine the contribution of OSM to epithelial repair. To this end, we will either overexpress OSM (colonic injection or hydrodynamic injection of recombinant OSM adenovirus) or inhibit OSM expression (blocking antibody and Knock out mice) and characterise the wound healing response using histology, spatial transcriptomic and immunohistochemistry.

Aim 2. Targeting Stromal OSMR expression in colitis associated cancer. We will first characterise the expression of OSM and its downstream signalling gene signature in bulk RNA and single cell RNA sequencing CRC data sets (in house and publicly available) as well as characterising the presence of neutrophils and stromal cells subsets in our own banked tissue of human CRC. The presence of OSM signalling and its associated immune-stromal network will be correlated with prognosis, mutations and CRC subsets. In addition, in vitro human organoid culture and primary stromal cells lines will be used to determine the function of OSM on CRC derived cell types. Finally, we will test the contribution of OSMR signalling (OSMRflox and STAT3flox) in cancer initiation and progression by targeting stromal cells using Platelet Derived Growth Factor (PDGFR)a and PDGFRb CRE^{ERT2} mouse strains and our models of colitis associated cancer induced by Dextran Sulfate Sodium and HhAOM.

Training opportunities

The successful applicant will benefit from a multidisciplinary supervisory team with access to cutting edge multiplex imaging and spatial transcriptomics platforms. 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 clinical samples. This project provides a broad training in cancer biology and immunology covering a range of cellular, molecular, and computational techniques. Students have access to cutting edge technologies such as disease positioned mouse models, *ex vivo* organoid models derived from human patients – co cultures systems.

Ideal student background: We are looking for a highly motivated and intellectually curious candidate with an interest in the intestinal tissue biology, epithelial regeneration, immune-oncology and translational medical research. The successful candidate is expected to be capable of working both independently and in teams, to have good communication skills and possess a general knowledge of the science supporting this project. They will also have had experience in a wet-based lab throughout their studies.

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35. Spatial transcriptomic analysis of the bone marrow landscape in blood cancer – Daniel Royston

Primary Supervisor: Daniel Royston
Additional Supervisors: Ros Cooper and Jens Rittscher
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract
Myeloproliferative neoplasms (MPN) are blood cancers characterised by overproduction of blood cells driven by well-defined driver mutations in *JAK2, CALR* or *MPL.* MPN patients are at increased risk of thromboembolic events with some patients also developing bone marrow scarring (myelofibrosis) or transformation to acute leukaemia, events associated with poor prognosis. Despite recent advances in our understanding of the common molecular abnormalities underlying MPN, it remains unclear why patients with similar driver mutations and clinical characteristics at diagnosis follow divergent disease trajectories. However, it is increasingly appreciated that perturbations in the bone marrow microenvironment and subsequent interactions with neoplastic haematopoietic stem cells (HSCs) are critical for disease initiation in MPN and influence disease and progression. Relatively little is known about the specific spatiotemporal relationships between these cell populations in the marrow of MPN patients. We have therefore developed and refined in-situ spatial transcriptomic (ST) approaches with particular focus on bone marrow tissue sampled from blood cancer patients. This allows high resolution detection and characterisation (phenotypic and genotypic) of individual stromal, immune and haematopoietic cell populations in the marrow of intact tissue biopsies. We will refine and develop computational / bioinformatic approaches to analyse and integrate this ST data, and integrate with recently developed image analysis / AI-powered tools designed to interrogate the morphological characteristics of the bone marrow in health and disease. Outputs from this work will be cross-validated using protein-based approaches including multiplex immunofluorescence and immunohistochemistry across larger clinical cohorts. This project aims to identify markers of early-stage disease progression in MPN suitable for translation to the clinic. It will also support ongoing efforts to validate and inform the search for novel therapeutic targets in MPN and related blood cancers.

Research objectives and proposed outcomes
RO1 - Extend and refine recent pilot ST analysis of bone marrow trephine (BMT) samples in MPN

- Employ recently developed strategies for handling and processing BMT specimens to expand and enrich the latest pilot ST data to create comprehensive whole sample 'single-cell' annotations from locally derived MPN patient cohort
- Apply and develop advanced computational / bioinformatic approaches to quantitatively capture key features distinguishing normal and diseased marrow microenvironment in MPN. These annotations will be validated against both conventional morphology and immunophenotyping.
- Integrate ST findings with those of established bone marrow stromal and immune MPN scRNA-seq datasets from collaborating groups to provide comprehensive annotations of spatially resolved single cell transcriptomic data.

RO2 - Develop computational methods to integrate the ST findings from RO1 with complementary image-analysis based descriptions of established pathological features in MPN

- Utilise our recently developed AI-based megakaryocyte and fibrosis detection and quantitation approaches to characterise associated ST-derived cell microenvironment signatures developed under RO1

RO3 - Establish and support new H+E-based algorithms trained on the integrated ST-derived morphological features (e.g. megakaryocyte and fibrosis) established under RO2 and refine existing AI-based algorithms to support improved diagnosis in MPN.

- Compare the cell signatures of early and advanced morphological abnormalities across important MPN disease subtypes and develop computational models to cluster key cellular and stromal features that are shared or restricted to MPN subtypes
- Train and validate H+E-based algorithms to identify and screen for morphological 'signatures' of early fibrosis and those predictive of disease progression using archival MPN patient samples, with line of sight to large scale clinical validation.

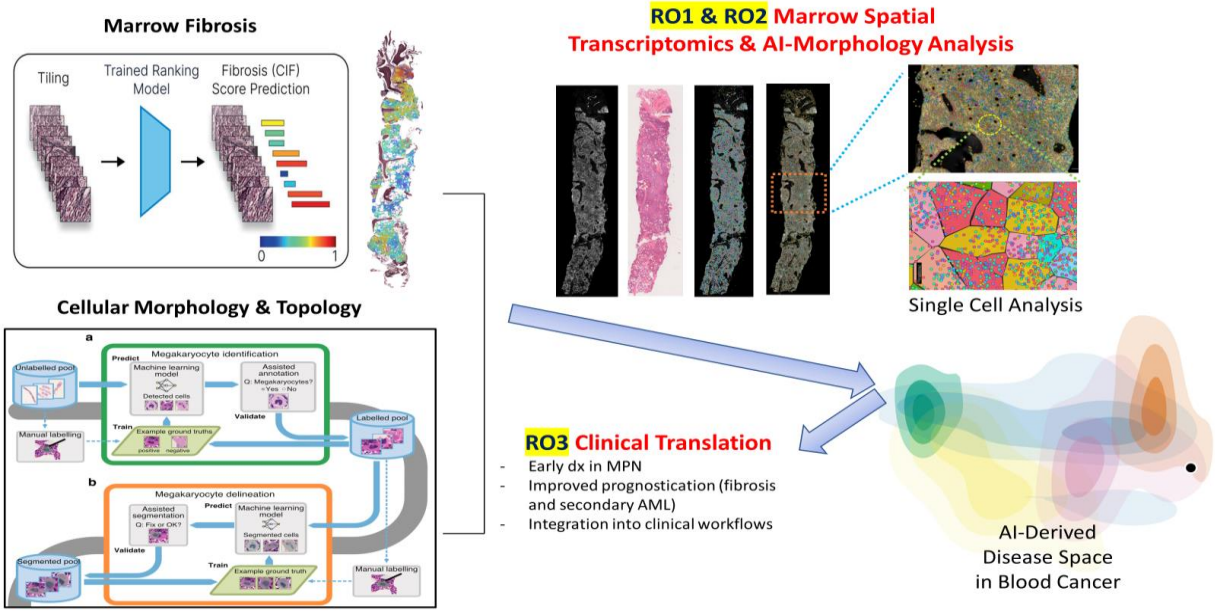

Figure 1. Overview of project workflow

Translational potential

The motivation behind this project is to address areas of unmet clinical need in the assessment of MPN patients, with focus on improving the accuracy and consistency of bone marrow biopsy interpretation and systematically characterising pathological features linked to disease progression in MPN. This work will strengthen and extend our group's commitment to develop personalised diagnostics in blood cancer, with line of sight to the development of validated algorithms suitable for integration into routine clinical reporting. The experimental design and objectives align with the goals of blood cancer patient advocacy groups and established funding bodies with whom we have ongoing support, including Blood Cancer UK, Cancer Research UK, MPN Voice and the MPN Research Foundation. To ensure transparency and maximise the clinical relevance of our work, members of our group regularly contribute to patient and public involvement (PPI) activities coordinated via the Oxford Blood Group.

Training Opportunities

In addition to the generic training opportunities offered by the Oxford Cancer Centre, DPhil students will be trained in a wide range of tissue diagnostic and analytical techniques including conventional microscopy, immunohistochemistry (IHC) / immunofluorescence (IF) microscopy, and spatial transcriptomics. They will also be trained in the analysis of 'omic' data including single cell and bulk transcriptomic datasets. This will involve supervised training in the use of specialist software and incorporate methodologies designed to analyse and

integrate multi omic data from patient samples. Training will be supported by collaborators spanning multiple research themes and clinical / academic departments within the University and NHS. Successful applicants will also participate fully in Prof. Rittscher's successful student training programme at the IBME, incorporating weekly lab meetings.

Ideal student background: The project would suit a clinical or science graduate with a background in computational biology or bioinformatics and big data. Familiarity with the principles of digital image analysis / AI would be desirable but is not essential.

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Artificial intelligence-based morphological fingerprinting of megakaryocytes: a new tool for assessing disease in MPN patients. *Blood Adv. 2020.*

Return to Projects list

36. Restricting the emergence of drug resistance in prostate cancer: injectable polymeric microparticles for the localised and sustained release of androgen receptor antagonists – Molly Stevens

Primary Supervisor: Molly Stevens
Additional Supervisors: Ian Mills
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract.
In the UK, about 1 in 8 men will get diagnosed with prostate cancer in their lifetime. Prostate cancer is a hormone-dependent malignancy where the growth and survival of cancer cells are driven primarily by androgens. As such, androgen receptor (AR) antagonists are a cornerstone of prostate cancer treatment. Clinical trials, such as STAMPEDE, have shown significant survival benefit when AR antagonists are administered in the earlier stages of the disease.[1] However, the use of AR antagonists is associated with the emergence of drug-resistant phenotypes and significant systemic side effect.[2] In this project, we aim to develop locally injectable and biodegradable microparticles for the sustained and localized delivery of commonly used androgen receptor antagonists. We will assess the impact of this drug delivery strategy on the development of drug-resistant phenotypes, as well as its safety and efficacy, using pre-clinical cancer models.

Research Objectives and proposed outcomes

1. Development of the drug delivery system. Within the Stevens Group, we are developing a novel drug delivery system using microparticles made from clinically approved and generally recognised as safe (GRAS) surface-eroding biodegradable polymers.[3] Unlike bulk-erodible polymers, like PLGA, these ensure a steady drug release over time. The drug-loaded microparticles are produced via microfluidic-assisted emulsion, offering a scalable and modular platform for fabricating highly monodisperse and reproducible microparticles. The degradation rate can be precisely tuned by adjusting the polymer composition, crosslinking degree, and microparticle dimensions. Preliminary data show that small hydrophobic drugs, similar in structure to clinically approved AR antagonists, distribute uniformly within the polymer matrix during fabrication and are released with quasi-zero-order kinetics. The degradation time can be tailored between 3 and 24 months. We aim to optimise drug loading and degradation profile for the localised, sustained and controlled release of AR antagonists, so to maintain therapeutic levels over the desired period. This approach aims to enhance therapeutic efficacy while reducing systemic side effects and the emergence of drug-resistant phenotypes.

2. Evaluate the impact of the proposed drug delivery system on the emergence of drug-resistant phenotypes in pre-clinical prostate cancer models. Professor Mills' team has notable expertise in constructing pre-clinical prostate cancer models and utilising them to unravel cancer progression, particularly focussing on the emergence of drug-resistance phenotypes such as neuroendocrine cancer cells. These transition states can be identified by gene signatures associated with the activation of transcription factors that drive lineage state change.[4,5] We will use chromatin immunoprecipitation and sequencing and transcriptomics to compare the effect of different drug delivery strategies on the emergence of the neuroendocrine drug-resistant phenotype in a range of pre-clinical prostate cancer models, including prostate cancer cell-lines, patient-derived organoid and *in vivo* syngeneic mouse models representative of both the hormone-sensitive and hormone-insensitive stage of the disease. We will compare our proposed delivery strategy to both conventional AR-antagonists delivery and bipolar androgen deprivation therapy – where patients receive AR-antagonists and high-dose testosterone in alternating treatment cycles - currently in clinical trial.

3. Evaluate safety and efficacy of the proposed drug delivery system in pre-clinical prostate cancer models. Responses in *in vitro* models will be assessed using viability assays and cell death assays to define

short- and long-term cytotoxicity and dose responses. Having defined sub-toxic long-term dose ranges, we will assess efficacy in these conditions based on the suppression of androgen receptor activity (transcriptionally and proteomically). Secondarily we evaluate the emergence of neuroendocrine phenotype, by assessing transcript and protein changes using RT-PCR and Western blotting. Equivalent measurements will be conducted *in vivo* using distinct approaches – volumetric measurements, bio-luminescent imaging of tagged engrafted lines and whole-body weight measurements over time. In addition, we will perform multi-timepoint tumour harvesting and downstream processing for transcriptomic and protein measurements.

This joint DPhil project will bring together Professor Stevens' expertise in drug delivery and advanced therapeutics with Professor Mills' proficiency in cancer biology. This synergy is designed to enhance the development of innovative treatment strategies for prostate cancer. Additionally, we plan on engaging clinical advisors who specialise in drug-resistant prostate cancer, who will provide critical insights and bridge the gap between research and clinical application.

Translational potential of the project.
This project spans bioengineering, cancer biology, and clinical oncology to innovate prostate cancer treatments and streamline their clinical translation. By developing locally injectable, biodegradable microparticles for sustained delivery of AR antagonists, it addresses a critical need in prostate cancer therapy. Sustained release of AR antagonists can help overcome drug resistance and enhance treatment effectiveness. Additionally, localized delivery minimizes systemic side effects, improving tolerability, patient compliance, and quality of life. Since both AR antagonists and polymer matrices proposed for the project are either clinically approved or GRAS, translation into practical medical applications would be significantly expedited.

Training opportunities
1. **Polymer synthesis.** Depending on the candidate's interest and expertise, they will have the opportunity to receive specialised training in polymer chemistry for the synthesis and fine-tuning of the polymer matrix. They will be guided and supported as necessary by experienced chemists within the Stevens group.
2. **Microparticles fabrication and characterisation.** The candidate will learn how to use microfluidic-assisted techniques to fabricate drug-loaded microparticles. Additionally, they will acquire skills to characterize these microparticles through various techniques, including but not limited to dynamic light scattering (DLS), scanning electron microscopy (SEM), degradation and release studies (biochemical assays, single-particle Raman spectroscopy).
3. **In vitro prostate cancer models.** The candidate will gain expertise in handling and characterising different preclinical prostate cancer models, from cell lines culture to patient-derived organoid preparation. The candidate will gain valuable expertise in advanced techniques in molecular biology and genomics, including but not limited to chromatin immunoprecipitation and sequencing (ChIP-seq), RNA sequencing (RNA-seq), and transcriptomics.
4. **In vivo prostate cancer models.** The candidate will undergo Personal License training to undertake cell-line engraftment (sub-cutaneously and orthotopically), and monitor changes in tumour volume, whole-body weight and murine health over time and in in vivo imaging.

The proposed DPhil project ensures an enriching academic experience within a highly multidisciplinary, collaborative, and translational environment. The student will not only acquire technical proficiency in both drug delivery and cancer biology, but also hone transferable skills typical of a doctorate programme, such as ethical research, data management, analysis and interpretation, science communication and many more. By working across disciplines and integrating diverse perspectives, the student will be primed to address complex issues effectively, making substantial contributions to both academia and real-world applications.

Ideal student background: While backgrounds in biochemistry or pharmaceutical sciences are preferred, this project welcomes individuals from all disciplines who possess a strong passion and inclination for multidisciplinary and collaborative research.

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37. Developing single-cell transcriptomics tools for PARP inhibitor resistance in BRCA1/2-deficient cells and tumours – Madalena Tarsounas

Primary Supervisor: Madalena Tarsounas
Additional Supervisors: Christiana Kartsonaki
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

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

a. Characterize the single-cell transcriptomic landscape of olaparib-resistant *BRCA1/2*-deficient cells in culture.
The scRNAseq technology will enable us to generate gene expression profiles of single cells and to identify cell sub-populations with specific transcriptional signatures. To achieve this first objective, scRNAseq will be carried out in populations of olaparib-sensitive and -resistant *BRCA1/2*-deficient cells, already generated in Tarsounas lab. We will prepare libraries from each cell line, before and after olaparib resistance onset, to sequence between 7,000 and 10,000 cells using the standard protocol of the Chromium Single-Cell 3' gene expression profiling solution (10x Genomics).
Unsupervised clustering approaches will be developed to classify cells into sub-groups with specific signatures (e.g. immune response, metastasis etc.) and to monitor cell dynamics using algorithms for pseudotime analysis. We will apply this combination of analytical approaches to the cell lines that are sensitive or become resistant to Olaparib, and anticipate that this will allow us to identify cell clusters with unique patterns of gene expression, which could 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 model's 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.

Translational potential of the project
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.

Ideal student background: This project would be suitable for a student with a strong interest in bioinformatics, statistics and cancer biology. Experience in using statistical software such as R, other programming languages, or command line tools would be highly desirable. Familiarity with statistical concepts, computational biology resources, handling complex datasets and wet lab experience is desirable. Ideally the candidate would have some formal training in both biology or a related discipline and statistics or a related numerate discipline. A strong interest in developing strong programming and bioinformatics skills as well as knowledge of cancer genomics and biology is essential.

38. Elucidating the role of trans-lesion synthesis DNA polymerases in mutational processes and therapy resistance – Marketa Tomkova

Primary Supervisor: Marketa Tomkova
Additional Supervisors: Ian Tomlinson
Eligibility: Track 3 and Track 4 applicants are eligible to apply for this project

Abstract
One of the open questions in cancer mutagenesis is what proportion of the cancer-causing mutations are due to errors made by DNA polymerases during DNA replication.[1,2] Understanding the sources and mechanisms of cancer-causing mutagenesis is critical for identifying effective preventive strategies, predicting personalised response to therapy, and designing novel therapeutics.

Trans-lesion synthesis polymerases (TLS) enable cells to replicate damaged DNA that would otherwise lead to replication fork collapse and cell death. However, TLS polymerases are inherently error-prone and introduce new mutations into the DNA, potentially contributing to the development of cancer. The aim of this project is to elucidate the role of the error-prone TLS polymerases in mutational processes, using computational genomics combined with wet-lab approaches. Second, we aim to develop computational tools predictive of response to therapy, as TLS polymerases contribute to resistance to chemotherapy by bypassing replication-blocking lesions induced by chemotherapy such as cisplatin[3–5].

Research objectives and proposed outcomes
Aim 1: Computational genomics approach to identify mutational signatures of TLS polymerases
Carcinogens and mutagenic processes leave distinct footprints in DNA, detectable using the computational approach of mutational signatures6. Remarkably, aetiology of nearly one-third of the mutational signatures in cancer patients is unknown, and there are open questions about the exact molecular mechanisms in many of the described mutational signatures.7 Understanding these mechanisms is important for prevention (e.g., to know how we can change our lifestyle to avoid cancer), predicting risk and personalised therapy (e.g., using the signatures as biomarkers), and designing novel therapeutics (e.g., based on synthetic lethality).

The first aim of this project is to identify the contribution of TLS polymerases to the previously detected mutational signatures and to develop refined TLS signatures using novel computational approaches by utilising additional genomic features and other data (including TLS gene expression, locations of regions where TLS polymerases get recruited, and specialised datasets of samples deficient in one TLS polymerase and compensated by other TLS polymerases). Candidate mutational signatures of TLS polymerases will be identified, comparing traditional ways of de novo signature extraction, with novel approaches, such as deep-learning-based methods.

Aim 2: Direct in vitro and in vivo measurement of error-signatures of TLS polymerases
One of the challenges in studying DNA polymerase errors is that they are very hard to measure. We have recently developed a specialised technique called Polymerase Error Sequencing (PER-seq) to detect the errors made by DNA polymerases in single molecules in vitro (cell-free) in unprecedented detail. Here, we will apply PER-seq to selected TLS polymerases to obtain direct measurements of their error signatures, unobscured by DNA repair or other complex cellular processes. We will then complement this with sequencing of TLS-mutant/overexpression mouse and/or cellular models (Tomlinson lab) and analysis of sequencing data from other previously published resources.

Aim 3: Prediction of resistance to therapy due to TLS polymerases
Finally, we will evaluate the potential of these signatures to predict survival and resistance/response to treatment using data from cell-lines9, recently cleaned and curated TCGA Resource10, Genomics England, Hartwig Medical Foundation, ICGC and focussed datasets such as the SCOT clinical trial, and GDSC. Selected candidate predictions may be validated experimentally.

The expected outcomes of this project include (a) mutational signatures of TLS polymerases with support in human cancer data, in vitro, and in vivo models, (b) novel computational methods for signature detection, (c) mechanistic understanding of TLS role in mutagenesis, and (d) biomarkers of TLS-based therapy resistance.

Translational potential of the project
TLS polymerases enable bypass of chemotherapy-induced DNA damage, leading to therapy resistance. TLS polymerases thus represent an attractive target for sensitizing cancer cells to genotoxic therapies. Indeed, inhibitors of TLS or their protein-protein interactions show promising synergy with therapies such as cisplatin, temozolomide, PARP inhibitors, and others 3–5,11,12. It is thus of increasing importance to understand the mechanisms and extent of TLS contribution to chemoresistance and to develop biomarkers of resistance due to TLS polymerases. The signatures of individual TLS polymerases will elucidate which TLS polymerases are involved in resistance to different therapies, and will help to predict which patients would benefit from TLS inhibitor-based treatment. Finally, the aims 1 and 2 are also expected to elucidate the mutagenic role of TLS polymerases in genesis of different cancer types, with potential implications for cancer prevention.

Training opportunities
The student will have the opportunity to learn transferable skills, including big data analysis, data visualization, machine learning and potentially deep learning, statistics, high-throughput computing, bioinformatics, and computational genomics, including integration of large sequencing genomic, epigenomic, transcriptomic, and other data set. The interdisciplinary nature of the project will provide opportunity to also gain laboratory skills in a range of molecular biology techniques, PER-seq, and other methods. Support will be provided to develop soft skills in presenting, writing, critical thinking, experimental design, and networking within the Oxford scientific community and at conferences.

Ideal student background: The project is most suitable for students with interest in cancer genomics and (a) either some prior knowledge of programming (e.g., Python, R, MATLAB, etc.), or (b) wet-lab experience, or both.

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Return to Projects list

39. The role and mechanism of highly variable genetic factors in cancer risk and prevention – Ian Tomlinson

Primary Supervisor: Ian Tomlinson
Additional Supervisors: Christiana Kartsonaki
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract
Identifying and characterising cancer risk factors is important for identifying individuals at increased risk of disease and working out how those risk factors operate to increase the chance of disease. Ultimately, that knowledge feeds through into cancer prevention. Genetic risk factors are largely unavoidable, but they can be mitigated. Since genetic factors provide particularly strong evidence for the mechanisms underlying carcinogenesis, they have great potential for developing new prevention measures, such as chemoprevention that could be used in the same way as cholersterol-reducing and blood pressure-lowering agents are widely used to reduce the risk of cardiovascular disease. Whilst studies over the last 10 years have made large inroads into identifying inherited cancer risk factors, much remains unknown, in part because the techniques used have not been able to analyse some types of genetic variation on a large scale. Nevertheless, we can now begin to study those variants, such as short or complex repeat sequences, owing to the growing number of cancer patients whose whole genomes have been sequenced. This project will search for inherited variation in repeated DNA sequences that increases the risk of colorectal and other cancers. The same sequences may also undergo acquired changes as tumours develop and help to drive cancer growth. The project will include computational analysis of large human data sets with genetic data (e.g. 100,000 Genomes, UK Biobank) and laboratory analyses. The balance between these types of work is flexible.

Ultimately, the project should identify mechanisms of tumour growth and strategies to counter those mechanisms. The project is novel, in that very little work has been performed in the specific area to date, but builds on multiple related studies performed by the supervisors that have identified >200 cancer-associated genes or polymorphisms to date.

Research objectives and proposed outcomes
Background
Many types of repeat sequence are present in the human genome and in some cases, that variation can affect the expression of genes involved in increasing cancer risk. The simplest repeats are arguably short tandem repeats (STRs, microsatellites) that comprise strings of repeated DNA bases. The length of the repeat varies considerably and studies have shown that this can affect gene expression and hence influence the risk of disease. However, we have not been able to study these sequences at scale in the past, because whole genome sequencing (WGS) data are needed for several thousand patients and controls. We can now start to address the issue of microsatellites and cancer risk. In short, we can perform a hypothesis-free search for associations between microsatellite allele lengths and gene expression in colorectal tissues (normal and tumour), and then measure the lengths of such microsatellites in large sets of cancer cases and population controls. Significant associations will identify cancer risk polymorphisms and can lead to additional functional studies in human cancers prone to acquire somatic microsatellite mutations and of the genes targeted by the change in gene expression. Strategies to use the data to prevent cancer will be developed for the longer term.

Project
1. Use existing information to identify locations of variable microsatellites in human genomes.
2. Develop simple methods to identify the lengths of the two alleles from short- and long-read sequencing data of constitutional DNA.

3. Use public and in-house reference data to link variation in microsatellites to expression of nearby genes in normal tissue and tumours
4. Identify a set of microsatellites associated with differential gene expression or function (e.g. polymorphic microsatellites or short repeats can occur in coding sequences or influence gene expression by affecting transcription factor binding)
5. Test those microsatellites for inherited differences between large sets of colorectal cases and controls, thus identifying cancer risk factors.
6. Extend the search to other types of repeat sequence or perform laboratory studies of selected target genes, depending on data obtained and preferences of the DPhil student.
7. If time permits, expand the analysis to include the role of microsatellite mutations acquired by caners, especially those with defective DNA mismatch repair that have an increased rate of microsatellite mutation.

Translational potential

The project will improve understanding of how this type of genetic variation contributes to cancer risk and to identify somatic driver mutations caused by STRs for different cancer types. In the long term these may contribute to predicting risk of cancer and in the development of treatment and prevention strategies.

Training opportunities

The student will receive full training in statistical and bioinformatics methods, in genetic epidemiology and genomics, as well as software commonly used in such analysis, such as R, Unix and command-line tools. Access to large genomic data sets will also require training in the use of secure research environments, e.g. UK Biobank, Hartwig Foundation, Genomics England. Training will also be provided, if desired, in relevant laboratory methods.

Ideal student background: None specifically required, but some computational background, interest or ability would be helpful.

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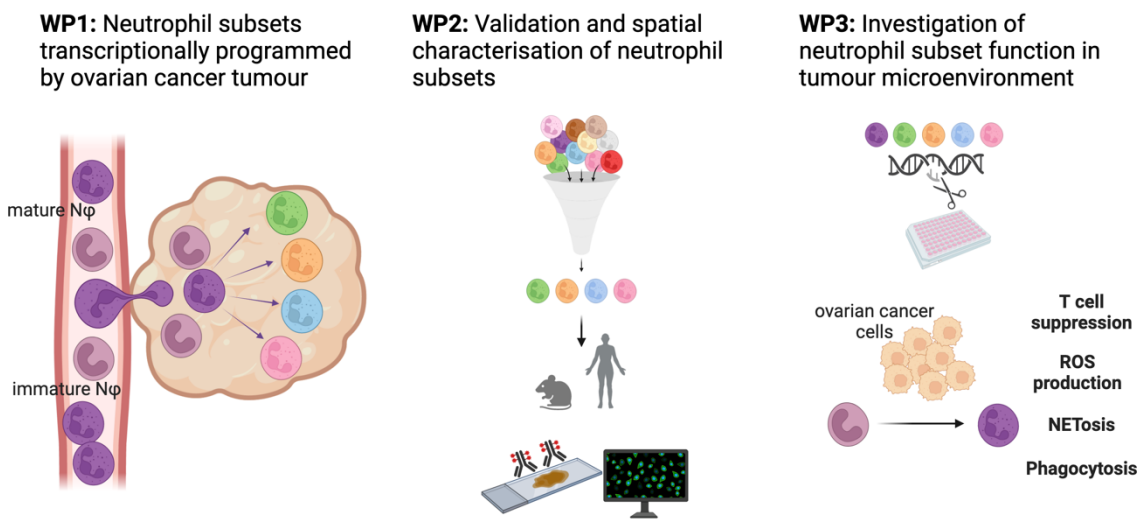
40. Spatiotemporal heterogeneity of neutrophil subsets in ovarian cancer – Irina Udalova

Primary Supervisor: Irina Udalova
Additional Supervisors: Sarah Spear
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract
Ovarian cancer is the 6th most common cancer in women, of which over 90% cases are epithelial high-grade serous carcinoma (HGSC) (CRUK). The majority of women are diagnosed with HGSC at stage 3-4 where the 5-year survival remains poor at 15-25%. HGSC disseminates throughout the peritoneal cavity generating secondary tumours, including on the omentum. Patients rely on surgery and chemotherapy to achieve remission, however even with PARP-inhibitor maintenance, acquired resistance is common and relapse rates are high. Therefore, approaches that circumvent therapy resistance are urgently needed. Neutrophils are the most abundant immune cell circulating in the human body and are actively recruited in ovarian cancer (1,2). A high neutrophil to lymphocyte ratio is a predictor of poor prognosis in ovarian cancer patients (3). As immune checkpoint blockade, targeted to lymphocytes, has shown no efficacy at clinical trial (4,5), it is critically important to understand how neutrophils might contribute to the progression of ovarian cancer. Neutrophils are typically seen as transcriptional inactive cells, with a short life-span that are rapidly recruited to inflammatory sites. However, recent work by us and others in the context of inflammatory disease have revealed neutrophils are heterogenous dynamic cells, transcriptionally imprinted by their microenvironment (6,7). Mounting evidence shows that the HGSC tumour microenvironment (TME) also modulates neutrophil function. Ovarian cancer-derived neutrophils have an extended life-span and immunosuppressive phenotype (2). They can produce neutrophil extracellular traps (NETs) and enhance tumour cell attachment (1). On the contrary, they can also upregulate costimulatory molecules and stimulate T cell IFNγ production (8). Pan-targeting neutrophils both improves and worsens survival, demonstrating neutrophil functional heterogeneity exists. To date, no *in vivo* transcriptional and functional characterisation of neutrophil subsets in ovarian cancer tumours has been performed.

Research objectives and proposed outcomes



We hypothesise that neutrophils are transcriptionally reprogrammed by the HGSC TME leading to the development of distinct neutrophil subsets. Understanding the function of these subsets in the TME will uncover new avenues for targeting neutrophils.

Aims:

1. Characterise the neutrophil subsets within the HGSC TME and identify molecular pathways.
2. Unravel the spatial organisation of these neutrophil subsets in murine and human tumours.
3. Examine the role of neutrophil-specific molecular pathways in driving neutrophil functions within the TME.

Outcomes: This project will generate a blueprint of neutrophil molecular heterogeneity in ovarian cancer and uncover transcriptional networks that control neutrophil functions. We will develop novel neutrophil knockouts to validate the function of these subsets in disease progression. Targeting these subtypes remains completely unexplored as a therapeutic opportunity. These methods can uncover new avenues for targeting neutrophils that can be replicated in other cancers. (ii) Collaborations: This project underpins a newly set up collaboration, between Oxford and Imperial College. It will combine the unique expertise of Prof Udalova (Oxford) in the transcriptional networks that control the heterogeneity and function of neutrophils with an expertise in models of ovarian HGSC of world-leading ovarian cancer researcher, Prof McNeish. Dr Sarah Spear (RcoI) is a postdoctoral researcher specialised in characterising myeloid cells in both inflammation and TMEs. Her research interests and pilot data have laid a foundation for this proposal, bringing together Udalova team and McNeish expertise.

Translational potential of the project

Immune checkpoint blockade, targeted to lymphocytes, has shown low efficacy in ovarian cancer at clinical trials. T cell immunity, which is beneficial in tumours, is undermined by immunosuppressive myeloid cells. Thus, it is critically important to understand how these cells, and specifically less studied neutrophils, might contribute to the progression of ovarian cancer. This will help shaping specific therapies targeting neutrophil subsets. We have already identified a number of regulators that play a critical role in mediating neutrophil recruitment and their differentiation, as well as in effector functions, in inflammatory settings. More will be identified during the course of this project, specific to the ovarian cancer development. The inhibitors of these regulators or their activation pathways may prove beneficial for inflammation-induced cancer.

Training Opportunities: The student will be trained in the models of ovarian cancer as well as cutting-edge microscopy and spatial transcriptomics approaches to define the localisation of myeloid cell subsets within the tumour microenvironment.

Ideal student background;The applying student would be eager to learn new techniques and models as well as able to work independently and in collaboration. They ideally would have an immunology or genomics background and developed interest in cancer, innate immunity and mucosal immunology. A vibrant collaborative group is awaiting them and there is an opportunity to learn various cutting-edge techniques as well as basing new discoveries on well-established models in the group.

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41. Can wearable sensors improve risk assessment before, and recovery after, major cancer surgery? – Stefan Van Duijvenboden

Primary Supervisor: Stefan Van Duijvenboden
Additional Supervisors: Sheraz Markar
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract
Background Peri-operative morbidity and mortality in patients undergoing major cancer surgery remains an important health problem. Consequently, accurate prediction of peri-operative morbidity and effective assessment of recovery in patients undergoing surgery are two key clinical challenges. Currently, cardiopulmonary exercise testing (CPET) has shown to be useful to assess fitness before major surgery[1]. However, due to its restricted availability, CPET is often limited to a select subset of patients and specific high-risk procedures. The increasing affordability and widespread use of wearable sensors offer new opportunities to remotely monitor physical and cardiac activity. These devices are cost-effective, objective, minimally invasive, and capable of capturing patients' everyday activities[2]. This has raised the question whether these devices might be useful to improve risk assessment before, and recovery after, major cancer surgery, especially since preliminary evidence has shown that device-measured activity at home correlate with key CPET parameters[3].

Knowledge Gaps Currently, the implications of wearable monitors in peri-operative risk assessment in major cancer surgery are largely unknown due to the absence of good quality validation datasets with wearable and longitudinal outcome data. In addition, addition of signal modalities including heart monitoring and more advanced signal processing methods to measure different activity patterns, such as sleep duration, activity have high potential to further improve assessment of fitness.
Project Contribution The proposed project aims to systematically evaluate the utility of wearable sensors in assessing perioperative risk and recovery in patients undergoing esophageal and gastric cancer surgery. This involves establishing a multicentre longitudinal cohort, collecting data on wearable activity and heart rate during patients' daily activities, alongside formal CPET assessments and longitudinal outcome data.

Research objectives

1. How well can wearable sensors characterise physical fitness in patients undergoing surgery for oesophageal and gastric cancer?
2. What is the impact of major cancer surgery on patient relevant physical activity and sleep outcomes?
3. Can wearables improve the prediction of postoperative complications at 30-days after surgery?

Translational potential of the project.
The potential of this project to meaningfully impact patient care is tremendous, as at present the assessment of patient preoperative risk and counselling patients around postoperative recovery is severely limited by a lack of objective quality data.

The proposed body of research will include patients from across 10 high surgical volume centres performing oesophageal and gastric cancer surgery in the UK, and has been endorsed by the Association of Upper Gastrointestinal Surgeons for Great Britain and Ireland (AUGIS). Therefore this study will be able to test the clinical utility of accelerometer and ECG monitoring in a longitudinal fashion during oesophageal and gastric cancer treatment, starting before chemotherapy (close to diagnosis), after chemotherapy and before surgery, and at 30- and 90-days after surgery. The research will be novel, in creating a granular dataset to assess postoperative recovery in a large cohort of Western population patients receiving oesophageal and gastric cancer surgery. This will allow clinicians in the future to appropriately counsel patients before surgery around their risk of postoperative complications and also what their long-term recovery is likely to be.

Given the support we have received for this research from AUGIS and 10 UK surgical centres, the results of this research will be of great interest to the wider oesophago-gastric community, and thus will facilitate a more direct pathway to clinical implementation following completion of the research.

Training opportunities

Experimental Design and Validation

The proposed project will provide a unique opportunity for the student to gain experience in setting up large multi-centre experimental studies. This includes training to design robust experiments, conducting power analyses, and how to handle potential biases when carrying out large validation studies.

Data analysis and machine learning

A key objective of this work is to implement and adapt existing methodology to objectively measure behavioural activities from wearable sensor data, including accelerometers and cardiac monitors. The student will work in close collaboration with the Wearables Group in the Oxford Big Data Institute (Dr Stefan van Duijvenboden / Prof. Aiden Doherty), which has extensive experience in developing reproducible machine learning methods to robustly identify both behavioural and disease-specific phenotypes.

Statistical methods

The project offers opportunities to develop methods to account for the unique nature of wearable phenotypes in relation to health association and risk prediction analyses. Both clinical and wearable groups involved in this project are actively involved in large-scale wearable epidemiology studies across a diverse range of cohorts, providing opportunities for the student to gain experience in conducting association analyses.

Collaborations & translational research

The results of this research will be of great interest to the oesophago-gastric community. Opportunities to present the work at (inter)national conferences and actively discuss the work with experts in the field will provide new insights and prepare the student to work on future applications that aim to shape the future work on integration of wearable technology in cancer research.

Ideal student background: Student should have a clinical background and an interest in recovery from major cancer surgery. Ideally the student should have previously completed their basic medical degree and at least two years of clinical training. The student should have an interest in programming and an understanding of wearable utilisation in clinical practice.

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42.BLOod Test Trend for cancEr Detection (BLOTTED): an observational and prediction model development study using English primary care electronic health records data – Pradeep Virdee

Primary Supervisor: Pradeep Virdee
Additional Supervisors: Brian Nicholson and Eva Morris
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract
Blood tests are commonly requested in NHS General Practice. Blood tests might be ordered when the patient attends their GP with symptoms or signs, to monitor a known medical condition, or as part of a "health check". Some clinical guidelines for GPs include recommendations that they should investigate for cancer if a blood test is lower or higher than a normal level. These recommendations are only helpful for a small number of cancers, such as bowel or pancreatic. Over time, a patient can build up a sequence, or trend, of blood test results. This trend might tell GPs more information than single blood test results. For example, a small drop from a steady trend could be more useful than waiting for the blood test to drop below a fixed level. However, the research has not yet been done to tell us which approach is most helpful to find patients who need referral to hospital for cancer investigation. In this research, we will explore if blood tests trend can detect patients with cancer better than single blood tests and check which patient groups trend is more helpful in and for what cancers, with primary focus on digestive and blood cancers.

Research objectives and proposed outcomes
Background: A recent clinical review confirms that simple blood tests have an important role in identifying patients for cancer investigation [1]. However, analysis of National Cancer Diagnosis Audit in Primary Care data suggests that primary care investigations may delay referral [2]. Smarter use of blood tests to select patients for further cancer investigation could increase cancer yield and reduce unnecessary referrals. Our recent research highlighted that trends over time in serial blood tests could be more useful than single blood tests and non-specific symptoms to select patients for colorectal cancer investigation, with our our colorectal cancer prediction models having good predictive ability [3,4]. However, trends are subtle so difficult to spot and may exist for various cancers.
Aim: To utilise trends in blood tests from primary care for early detection of cancer.

Objectives: There are three main objectives:
1) *identify trends among repeated blood tests indicative of cancer* – the student will learn of smoothing techniques, such as LOWESS, to graphically describe trends in each blood test, both overall and by personal, clinical, and cancer characteristics (e.g. age, sex, comorbidity, diagnosis route, site, stage). Collaborators: the Big Data Institute will collaborate on data curation and understanding of electronic health records data.
2) *assess predictive ability of blood test trends for different cancer types* – the student will learn of dynamic models, which utilise repeated measures data for assessing clinical outcomes. These include statistical models, such as joint modelling. Collaborators: the Big Data Institute will collaborate on the interpretation of repeated measures data from national datasets.
3) *develop and test prediction models utilising blood test trend to optimise patient selection for referral* – the student will learn of the intricacies of developing and testing dynamic prediction models and their clinical application. Collaborators: the Big Data Institute will collaborate on the interpretation of results from national datasets.

Data: Data from ~28 million patients from the CPRD primary care database is available to develop the models. It includes information on patient characteristics, deprivation, blood tests, symptoms, medications, cancer

diagnosis, and other variables over 2000-2019. It is linked to the National Cancer Registration and Analysis Service, Hospital Episode Statistics databases, and Office of National Registration death database.
Outcomes: The main outcome will be prediction models that incorporate blood test trend for cancer risk. Outputs will include peer-reviewed journal publications for each objective separately and conference presentations.
Academic value: This research will develop an evidence base for blood test trend for cancer detection and inform clinical practice. The DPhil candidate will develop leadership and research skills in various areas, including primary care, electronic health records data, patient and public involvement, and more. The student will grow their academic publication record and research networks at courses and events. Collaborations in this research will provide direct access to further multidisciplinary teams to improve efficiency in conducting this research.

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43. Manipulating intratumoural dendritic cell fate to enhance anti-cancer immunity – David Withers

Primary Supervisor: David Withers
Additional Supervisors: Audrey Gerard
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract
Targeting the molecular pathways that restrain T cells has achieved striking results in the treatment of some cancers, particularly those where the tumours contain numerous mutations that can be recognised by the immune system[1-3]. However, despite improving control of these more immunogenic tumours, the majority of patients still fail to durably respond[4]. Furthermore, there are many common cancers for which immunotherapy has had minimal impact, even in the short term[5]. Here it is likely that the anti-tumour T cell response failed to either properly establish, or reach a threshold at which immune checkpoint blockade could enhance the response. This may reflect a limited number of mutations visible to T cells, dominant suppressive mechanisms or a combination of both these factors. **Understanding why the T cell response is impeded and identifying approaches to overcome this failure to generate robust anti-tumour immunity, remains vital to supporting the design of better treatments for cancer patients.**

Tumours excel at subverting the anti-tumour response through multiple mechanisms[6, 7], for example creating tissue niches that exclude T cells or inhibiting T cell functions to render these cells ineffectual. Furthermore, productive CD8 T cell responses are not generated in cellular isolation, rather, they require a supportive team of other immune cells, in which dendritic cells (DCs) are key[8]. Activated DCs carry tumour antigens to draining lymphoid tissue to prime and expand the effector T cells that traffic to the tumour and kill cancer cells[9]. Alongside this critical role in establishing the anti-tumour T cell response, it is now appreciated that interactions between responding T cells and DCs within the tumour itself, further regulate T cell effector functions[10, 11]. Thus activated DCs lie at the heart of anti-tumour response, controlling the magnitude and fitness of the response. **However, we recently discovered some activated DCs become trapped within tumours, rather than migrating to lymphoid tissues, resulting in the formation of a distinct intratumoural DC compartment that appears to be dysfunctional**[12]. Thus DCs join the expanding list of immune cells that can become corrupted by the tumour microenvironment. Determining how best to block the formation, retention or functions of these defective DCs presents an exciting new approach to enhancing anti-tumour immunity.

We hypothesise that cancers: a) exploit mechanisms that cause the retention of activated DCs within tumours, thus impeding T cell priming in draining lymphoid tissue and b) accumulate altered DCs that further impede intratumoural effector T cell function.

The overarching aim of this project is to understand the mechanisms controlling DC fate within the tumour and how this impacts support for intratumoural T cells**.** Deciphering how and why this occurs has the potential to support the design of immunotherapy combinations tailored to ensure robust T cell responses. This is of particular relevance to cancers characterised by a limited T cell infiltrate, which includes the majority of colorectal cancers[13].

Firstly, we will interrogate the functions of the activated DCs that become retained with tumours, exploiting photo-labelling approaches developed in the Withers Lab to specifically capture cells based upon time spent within the tumour microenvironment[14, 15]. While this work is focused on colorectal cancer, including the use of orthotopic models implanting mouse tumour organoids, the relevance of dysfunctional intratumoural DCs will be assessed across other cancer types using other models established in the lab. Building from detailed transcriptomic resources, DCs from photo-labelled tumours will be characterised by spectral flow cytometry and

compared with DCs in draining lymphoid tissue, including those activated DCs that have demonstrably migrated (utilising photo-labelling to track cells). We will functionally test the ability of these DC populations to prime CD4 and CD8 T cells *ex vivo* and assess their provision of costimulatory and coinhibitory ligands. Secondly, using cutting-edge imaging approaches, alongside established models that support targeted manipulation of tumour cells and the local stroma, we will investigate the cellular niches in which DCs reside and seek to define the microenvironment(s) in which DCs become trapped.

Informed by the cellular interactions observed, alongside leveraging scRNA-seq resources, we will explore the mechanisms that orchestrate DC retention (e.g. blocking Abs *in vivo* combined with photo-labelling and tracking of cellular egress). We will then look to more definitively test the most promising candidates through genetic approaches. Finally, we will aim to identify immunotherapy combinations that boost DC support for the T cell response and, when combined immune checkpoint blockade, drive durable and systemic protection. Using the array of tumour models established within the lab, robust anti-tumour immunity will again be tested *in vivo*.

Research objectives and proposed outcomes

The specific research objectives for this Project are as follows:

1. Investigate DC fate over time, specifically in models of colorectal cancer, but further compared to other cancer types to establish broader relevance and tissue/site specific cues.
2. Define the functional capacity of different intratumoural DC populations and the mechanisms through which these are orchestrated.
3. Determine the cellular niches in which DCs reside and test the role of interactions with different stromal compartments in mediating DC retention.
4. Develop approaches to manipulate intratumoural DC fate and enhance the anti-tumour response.

The principle outcome of this project will be detailed mechanistic insight into how fate and function of DCs is controlled within the tumour microenvironment, fundamental knowledge that underpins the success of anti-tumour T cell responses. We will identify approaches to manipulate DC behaviour and test combinations of immunotherapies designed to enhance the anti-tumour response in pre-clinical models of colorectal cancer. Collectively, this research will inform of potential therapeutic approaches to enhance responses in cancer patients currently in desperate need of better treatments.

Translational potential of the project

Combinations of immunotherapy, tailored to the type and stage of cancer, offer clear potential in refining the clinical impact of immune checkpoint blockade and improving cancer patient treatment. Rationalising these immunotherapies requires detailed mechanistic understanding of the critical steps that drive and sustain the anti-tumour response. This is a fundamental research project that aims to help address this critical knowledge gap and support the development of better therapies for colorectal cancer patients.

Training opportunities

This project will provide a wealth of training opportunities and is ideal for students wishing to develop expertise in studying immune responses to cancer utilising the most advanced *in vivo* models available. Extensive training in an array of cutting-edge experimental approaches (e.g. photo-labelling, conditional targeting of molecular interactions, fate-mapping) across multiple cancer models (intradermal, orthotopic, mouse-tumour organoid) will be provided. Transcriptomic approaches (e.g. scRNA-seq), alongside flow cytometry (including spectral flow) and imaging approaches (multiplex imaging including Cell DIVE, MIBIscope) will be used to interrogate immune responses, alongside *ex vivo* functional analyses. Collectively this will ensure comprehensive training in the core approaches required to interrogate immune responses, with appropriate appreciation of immune cell phenotype, spatial distribution, cellular interactions and functional relevance.

This research project benefits from close interactions with multiple other labs, both locally (Gerard, Coles, Leedham) and nationally (Zelenay). This project further complements the research focus of a Wellcome

Discovery Award (led by DRW) and the student will be fully integrated into the regular meetings underpinning this team research programme, coordinated between the Withers Lab and the labs of Menna Clatworthy and Sophie Acton (Discovery Award CoApplicants).

The Withers Lab is highly collaborative and adopts a team science approach. There is extensive technical support for the *in vivo* research and the lab has a long track record in providing a supportive environment in which post-graduate students flourish.

Ideal student background: The student requires basic knowledge in immunology. An enthusiasm and interest in cancer immunology is essential. Knowledge/experience in tumour immunology is obviously advantageous. Experience in the use of immune assays, alongside in vivo or ex vivo experiments is necessary. The student should be willing to work with mouse models of cancer.

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44. Developing a vaccine for ovarian cancer prevention – Nancy Zaarour

Primary Supervisor: Nancy Zaarour
Additional Supervisors: Ahmed Ahmed
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract
High-grade serous ovarian cancer (HGSC) is the most lethal histotype of ovarian cancer, largely attributed to late-stage presentation. with a lifelong risk, in some cases, exceeding 40%, prompting current clinical recommendations for prophylactic surgery by the age of 35, emphasizing a substantial need for the development of preventative strategies for ovarian cancer including vaccination. Accumulating evidence emerging over the past two decades strongly indicate that most HGSCs are derived from the fallopian tube. Moreover, frequent mutations in the *TP53* tumor suppressor gene, arise at the premalignant lesions called STICs in the fallopian tube (FT), the earliest step of HGSOC development, in nearly 100% of cases, leading to further oncogenic mutations. However, whether or not local immunity in the fallopian tube plays a role in modulating transformation or establishing serous ovarian cancer has remained unknown. Based on our recent findings, we have now discovered that tissue resident memory T cells (TRMs) that reside in non-cancerous fallopian tubes react to tumour organoids derived from omental metastases in the same patient. Moreover, these memory cells induce apoptosis in tumour-derived organoids indicating that they possess cytotoxic ability. In this project, we propose to use our established models of fallopian tube organoids, ovarian cancer organoids and epithelial-immune cell co-culture methods to test the hypothesis that a vaccine that we designed based on ovarian cancer tumour associated antigens (TAAs) and recurrent mutations could be effective in preventing early transformed cells in the FT . We believe that a vaccine targeting TRMs would be highly effective in constraining the local spread of STICs by maintaining an immune-equilibrium in the FT. We will undertake this essential pre-clinical work as a stepping-stone towards clinical testing of our proposed vaccine.

Research objectives and proposed outcomes
Aim 1): To evaluate the immunogenicity and memory recall of ovarian cancer specific peptides in patients/and Healthy individuals with potential to develop a specific T cell response against these peptides. To this end, T cells isolated from PBMCs, fallopian tubes and tumour infiltrating lymphocytes (TILs) will be interrogated for their ability to respond to our selected peptides.
Subaim1) using ex vivo assays, we aim to assess the immunogenic potential of our selected peptide pools on Naïve T cells from peripheral blood of healthy blood-donor females aged 30-40 (to mimic the target vaccine population).
Subaim2) To elicit a secondary recall response in patients with ovarian cancer: We will investigate whether these peptides can activate memory cells from non-cancerous fallopian tubes of ovarian cancer patients, exploring potential memory response for ovarian cancer antigens. Such memory could be acquired through prior exposure to premalignant lesions. We will also test TILs from the same patient for comparison.

Aim 2): Using our extensive experience in T-cell-organoid co-culture systems that we have recently developed in our lab, we will Test the hypothesis that either *in vitro* primed naïve T cells or induced memory cells are cytotoxic to autologous ovarian cancer organoids and early-transformed fallopian tube epithelial cells.

Proposed outcomes: (i) Testing the efficacy of cancer vaccines in preclinical models prior to clinical trials is essential for successful vaccine development. However, such testing is very difficult since animal models do not faithfully recapitulate human cancer-immune interactions. Previous work in our lab lead to the development of organoid models that faithfully represented non-cancerous fallopian tube epithelial (FTE) composition (1, 2), as

well as 3D cancer organoid systems that recapitulate the *in vivo* genomic and transcriptomic features of ovarian cancer (3). Along with our expertise in T cell immunology, a tailored method has been recently developed in our lab to maintain the phenotype and to expand newly identified subsets of T cells termed TRMs homing the FTE. We propose to use our established models of fallopian tube organoids, ovarian cancer organoids and epithelial-immune cell co-culture methods to allow for testing of peptides-primed T cells' cytotoxicity against patient-derived ovarian cancer organoids, allowing for the selection of peptides/epitopes for vaccine development. Therefore, facilitating a successful completion of the proposed work that has several promising proposed outcomes. First, at preventing the onset of ovarian cancer development, a significant decrease in the number of new cancer cases by targeting high-risk populations will be expected. Prevention of ovarian cancer development in a clinical trial that should be undertaken after completion of the preclinical work, by envisioning a vaccination of patients prior to surgery (reduction in size and prevalence of STICs in the vaccinated groups compared to non-vaccinated will be expected). Second, early elimination of precancerous cells, by priming/boosting the local and systemic immunity, TRMs in the FTE will recall a memory response, recognize, and destroy cancer cells harbouring these early mutations and preventing progression to fully established cancer. Third, providing a durable immune response, by establishment of a long-lasting immunity and generation of memory T-cells that remain vigilant and ready to attack precancerous cells that present these proposed TAAs and neoepitopes at very early-stage of ovarian cancer. Therefore, reducing mortality, need for surgery, chemotherapy, and radiation, leading to a better quality of life.

This studentship would greatly facilitate collaboration opportunities with clinicians, academics and vaccine experts for the students by providing financial support, opportunity to work closely with experienced scientists. It can provide invaluable guidance, enhance the quality of the research, and help students navigate complex academic challenges. Additionally, networking with other scholars in the field can lead to interdisciplinary research. In addition to the practical skills in immuno-oncology and vaccine development they can acquire, access to resources including tissue cultures, FACS facilities and data analysis, equipment for molecular and cell biology. It also offers the opportunity to develop other transferrable skills through access to courses offered by the Medical Sciences Division, the WIMM, and the wider University. It can allocate a more focused mentorship, research time and professional development. These benefits collectively contribute to producing high-quality, impactful research that advances knowledge in T cell immunology, sequencing technologies, bioinformatics, cancer biology and the most advanced preventative vaccine strategies.

Training opportunities

1. Clinical samples processing and isolation of primary immune cells from matched FTs, tumours, and blood samples.
2. Developing immunogenicity assays for TAAs and mutated peptides screening:
3. in vitro assays include T cell expansion, flow cytometry, cell sorting and ELISpot cytokine release assays.
4. In vitro Dendritic cells/T-cell coculture assays.
5. Generation of FT and tumour 3D organoids.
6. Cytotoxicity assays using T-cell organoids coculture methods, followed by live imaging (Incucyte).
7. CRISPR-Cas9 knockout and single nucleotide editing.
8. Single-cell sequencing

References

1) Hu Z, Artibani M, Alsaadi A, Wietek N, Morotti M, Shi T, et al. The Repertoire of Serous Ovarian Cancer Non-genetic Heterogeneity Revealed by Single-Cell Sequencing of Normal Fallopian Tube Epithelial Cells. Cancer Cell. 2020;37(2):226-42 e7.
2) Alsaadi A, Artibani M, Hu Z, Wietek N, Morotti M, Gonzalez LS, et al. Single-cell transcriptomics identifies a WNT7A-FZD5 signaling axis that maintains fallopian tube stem cells in patient-derived organoids. Cell Rep. 2023;42(11):113354.
3) Yang X, Artibani M, Jin Y, Aggarwal A, Zhang Y, Muñoz-Galvan S, et al. A 3D microtumour system that faithfully represents ovarian cancer minimal residual disease. bioRxiv. 2023:2023.07.15.549155.