DPhil in Cancer Science

University of Oxford

Non-Clinical

2025 Intake Project Booklet

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DPhil in Cancer Science– Non-Clinical 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 programme has four different tracks with two different pathways of entry. This booklet will focus on Tracks 3 and 4; non-clinical/fundamental scientists, who will **select their preferred research projects from this 'DPhil in cancer science – Non-clinical project booklet'**

Non-clinical/Fundamental scientists will enrol on a four-year programme with the first year consisting of two six-month rotational projects in different research groups. This provides students with a broad base of experience and the opportunity to explore different aspects of research prior to deciding on a final project for their second year onwards. All students are admitted directly to work under the supervision of a Principle Investigator (PI) which will change between the rotation projects. Once the final project is chosen, the relevant PI will be formally appointed as the DPhil supervisor.

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

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

All applicants will be judged on the following:

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

Funding

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

- **Application Tracks 3 and 4:** Four 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.

CRUK Black Leaders in Cancer DPhil Scholarship Programme
This year we are participating in CRUK's Black Leaders in Cancer programme. Please see our webpage for further information.

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.**

Please note, although you are choosing your preferred DPhil research projects, you will be required to undertake two of the rotational projects outlined during the first year before making a final choice.

Shortlisted students will be invited to interview in January. If successful, students will then begin their first year with two lab rotations based on project preferences selected at the application stage, before choosing one to complete a three-year DPhil. It is strongly suggested that students contact supervisors of projects they are interested in applying for prior to application.

Projects

Clicking on a project title below will take you to the relevant project page. The 6-month rotational project can be found at the end of each project page.

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

Primary Supervisor: Ivan Ahel
Additional Supervisors: Ahmed Ahmed
Eligibility: Track 3 and Track 4 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.

Rotational Project: Assessing the effect of ARH3 under- and overexpression on PARP inhibitor sensitivity/resistance in a series of model cancer cell lines

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. Ivan Ahel laboratory recently identified HPF1 protein as a novel interactor and critical regulator of PARP1 ADP-ribosylation activity upon DNA damage. 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). Crucially, the work also identified ARH3 as a hydrolase which specifically removes Ser-ADPr and further showed that Ser-ADPr is the major form of ADP-ribosylation following DNA damage. 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.

Based on these results, we hypothesize that ARH3 activity and protein levels affect sensitivity to PARPi, thus representing a predictor for the success of these therapies. 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. This project will assess how ARH3 protein levels in different cancer cell lines correlate with their sensitivity or resistance to PARP inhibitors currently used in the clinic. The student will have opportunities to train in cell culture approaches, western-blotting and well-established long-term cell survival assays.

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

References

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2. Reconstructing the non-genetic heterogeneity of ovarian cancer using organoid models for drug discovery – Ahmed Ahmed

Primary Supervisor: Ahmed Ahmed
Additional Supervisors: Lena Rai
Eligibility: Track 3 and Track 4 applicants are eligible to apply for this project

Abstract of the project

The 5-year survival rate for High Grade Serous Ovarian Cancer (HGSOC) patients remains less than 45% (https://www.cancerresearchuk.org/). Novel therapies such as PARP inhibitors as well as anti-angiogenic drugs have demonstrated remarkable response rates in subsets of patients, however, around 50% of these patients show intrinsic or acquired resistance. New targeted therapies based on the tumour characteristics of poor prognostic patients has the potential to achieve sustained long-term clinical response. Previously, our lab discovered the Oxford Classic (OxC), a deconvolution algorithm-based analysis of the expression of 52-genes in Serous Ovarian Cancer. A higher OxC-based EMT score correlated with worse overall survival in over 13 independent patient datasets. More recently, we identified pathways and factors contributing to the poor prognosis of OxC-EMT-high tumours. Notably, these tumours harboured a significantly higher number of pro-tumour M2-macrophages and fewer cytotoxic CD8-positive tissue resident memory T-cells (CD8+TRMs) compared to OxC-EMT-low tumours. Further characterisation of tumour cells as well as cells in their microenvironment using state-of-the-art multi-omics technologies can highlight mechanisms that promote disease progression whilst enabling the discovery of novel druggable targets. Furthermore, there are no known organoid co-culture models of Serous Ovarian Carcinomas that captures the non-genetic heterogeneity of the disease. Once established, such an organoid model can provide valuable mechanistic insights into the aetiology of treatment resistant of OxC-EMT-high tumours. Such organoid models will make an ideal platform to screen for novel as well as combination therapies. In this study we will (i) investigate functionally distinct subsets of cells within the tumour compartment and the tumour microenvironment of both OxC-EMT-high and OxC-EMT-low tumours, and (ii) develop patient-derived 3D organoid co-culture models that mirror the Oxford Classic phenotypic heterogeneity.

Research objectives and proposed outcomes

Previously, our lab discovered the 'Oxford Classic', a 52-gene panel that identifies the two epithelial cell types- Secretory and Ciliated cells, as well as four phenotypic cell states within the Secretory cell type, ie; EMT,

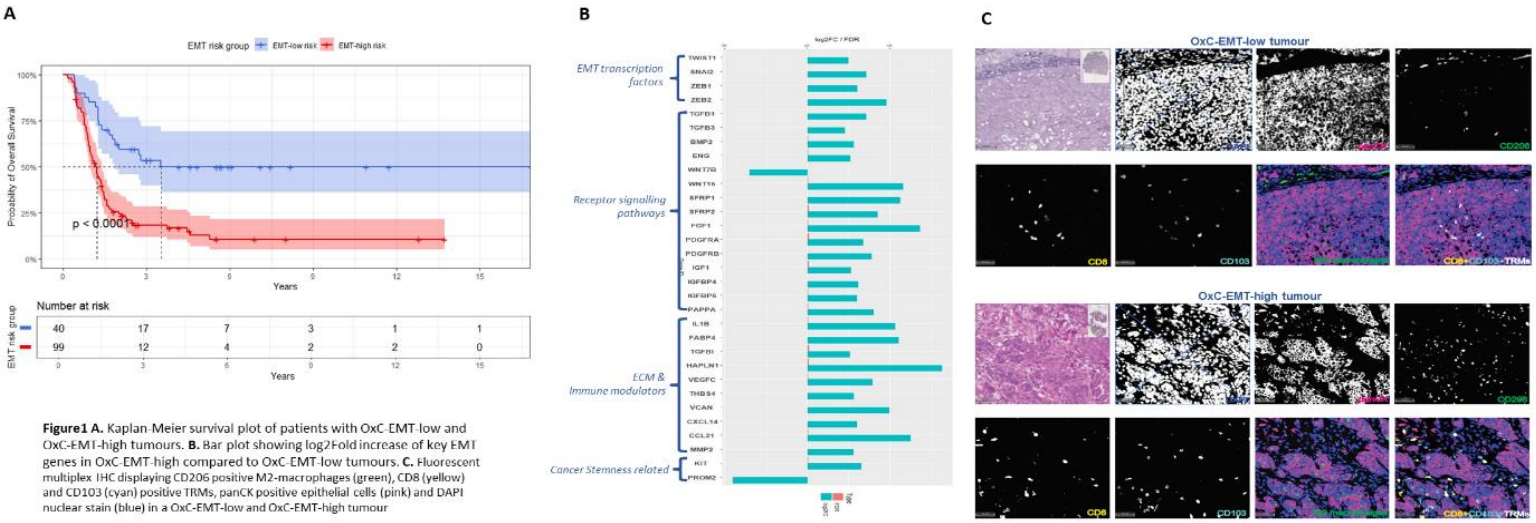

Figure1 A. Kaplan-Meier survival plot of patients with OxC-EMT-low and OxC-EMT-high tumours. **B.** Bar plot showing log2Fold increase of key EMT genes in OxC-EMT-high compared to OxC-EMT-low tumours. **C.** Fluorescent multiplex IHC displaying CD206 positive M2-macrophages (green), CD8 (yellow) and CD103 (cyan) positive TRMs, panCK positive epithelial cells (pink) and DAPI nuclear stain (blue) in a OxC-EMT-low and OxC-EMT-high tumour

Differentiated, Cell Cycle and KRT17[1]. Deconvolution of bulk tumour RNA sequencing data using the Oxford Classic demonstrated an association between a higher abundance of the Oxford Classic-based EMT (OxC-EMT) cell state and poor overall survival (Fig 1A). Our study also demonstrated that the OxC-EMT score was independent of other established clinical parameters such as age of the patient and stage of disease at diagnosis and residual disease following surgery[2].

Aim 1: To characterise the microenvironment of OxC-EMT-high tumours

Our latest study involving four independent patient datasets (manuscript in submission), has unequivocally confirmed that the OxC-EMT signature represents critical pathways characteristic of the epithelial to mesenchymal transition; which is a key feature of cancer cells that display metastatic potential and treatment resistance. However, characterisation of non-epithelial cells such as cancer-associated fibroblasts and immune cells present in the tumour microenvironment of OxC-EMT-high tumours can identify tumour promoting sub-populations, decipher cell-cell interactions as well as reveal novel therapeutic targets. In this study we will isolate by FLOW cytometry, tumour epithelial cells, immune cells, CAFs and endothelial cells from two each of OxC-EMT-high and OxC-EMT-low primary tumour tissues obtained from patients enrolled into the Gynaecological Oncology Targeted Therapy 01 study (REC number-11/SC/0014). We will then carry out single cell RNA sequencing (scRNAseq) of these cell populations to identify functionally distinct subsets of cells that may confer OxC-EMT-high tumours with treatment resistance. In order to spatially map the interaction between EMT tumour epithelial cells and other subsets of cells, we will use state-of-the-art Spatial Transcriptomics technology. Additionally, we will use cutting-edge Spatial Proteomics platform to spatially resolve protein expression of differentially expressed genes in OxC-EMT-high tumours, so as to confirm their host cell location and their therapeutic targetability. Apart from furthering our understanding of the factors that drive treatment resistance and cancer cell survival, this part of the project has a huge potential for novel drug development.

Aim 2: To establish a 3D organoid co-culture model of OxC-EMT tumours

Our study involving transcriptome analyses of OxC-EMT-high and OxC-EMT-low tumours identified pathways including TGFβ and PDGFR to be significantly upregulated in OxC-EMT-high tumour cells (Fig 1B). Our study also found profoundly higher abundance of pro-tumour M2-macrophages and significantly lower number of anti-tumour cytotoxic CD8+TRMs in these tumours (Fig 1C). Collectively, these findings suggest that OxC-EMT-high patients are more likely to respond to inhibitors of TGFβ/PDGFR, while a combination therapy that targets tumour epithelial cells as well as pro-tumour immune cells could elicit a sustained treatment response. However, functional experiments to validate the efficacy of novel drugs using organoid models that mimic the non-genetic heterogeneity of Serous Ovarian Cancers, remains to be carried out. Previously, our lab in collaboration with Prof. Hagan Bayley successfully established a 3D co-culture organoid model involving HGSOC tumour cells and fibroblasts using a novel microfluidics system[3]. We have also successfully co-cultured tumour epithelial organoids with certain immune cells. Based on our prior experience establishing organoid models, the findings from our latest study as well as those from AIM1, we will co-culture patient-derived tumour epithelial cells, tumour associated fibroblasts and immune cells in a culture medium that includes growth factors which support OxC-EMT-high tumours. These complex organoid models will then be used for testing the efficacy of EMT-targeting drugs as first-line of therapy and/or for targeting minimal residual disease (microscopic residual disease following treatment). The findings from this part of the study has enormous translational potential as EMT targeting drugs can provide HGSOC patients with a real chance to attain long term clinical remission.

Translational potential

The Oxford Classic-based EMT is a highly robust, independent prognostic biomarker of overall survival in HGSOC as validated in at least 13 independent patient cohorts. In the future, screening of HGSOC patients for

their OxC-EMT status and risk stratifying them into either 'OxC-EMT-high' or 'OxC-EMT-low', prior to commencement of treatment, can guide treatment decision making. Additionally, novel findings such as new druggable targets identified in this study, will be developed further in collaboration with commercial ventures. Moreover, Prof. Ahmed & Dr. Rai have recently received a £140K grant from the Wellbeing of Women to investigate OxC-EMT in the context of novel therapies.

Training opportunities

The DPhil candidate will be working in an interdisciplinary environment. They will be trained to carry out FLOW cytometry, single cell RNA sequencing, spatial transcriptomics, spatial proteomics, processing of fresh frozen tumour tissue from patients and to set-up organoid co-culture experiments. Students will also have the opportunity to train in bioinformatics tools to carry out single cell sequencing, transcriptomic, proteomic and other statistical analyses. Members of the lab regularly collaborate with Prof. Ahmed's immunotherapy company called SingulaBio, other commercial entities such as Miltenyi Biotech and other academic labs.

Rotational Project: Characterising Serous Ovarian tumours and their microenvironment for drug discovery

A dismal 5-year survival rate of less than 45% in High Grade Serous Ovarian Cancer (HGSOC) has been associated with recurrence of disease following treatment. The Oxford Classic-defined EMT (OxC-EMT) risk stratification of HGSOC patients clearly identifies OxC-EMT-high patients (~70% of patients) who have a significantly worse overall survival compared to patients who are OxC-EMT-low. Our latest study has found several key epithelial to mesenchymal transition pathways to be upregulated in OxC-EMT-high tumour cells, that are known to promote chemotherapy resistance and metastasis. Other studies have also demonstrated the role of non-epithelial cells in the tumour microenvironment such as cancer-associated fibroblasts (CAFs) and immune cells in promoting disease progression. A thorough investigation of the distinct cell populations in the tumour tissue of OxC-EMT-high and OxC-EMT-low patients, will help identify functionally distinct subsets of cells that support treatment resistance. These experiments can highlight novel therapeutic targets by identifying genes that are differentially expressed between OxC-EMT-high and OxC-EMT-low tumours. Additionally, this project will provide vital information regarding factors that are required to successfully co-culture HGSOC tumour epithelial cells, and components of the tumour microenvironment including CAFs and immune cells. Consequently, this study will lay the foundation for optimizing the medium for growing complex co-culture organoid models that mimic the non-genetic heterogeneity of Serous Ovarian Cancers.

In this study we will isolate by FLOW cytometry, tumour epithelial cells, CAFs, immune cells as well as endothelial cells from two each of OxC-EMT-high and OxC-EMT-low tumour tissue obtained from HGSOC patients enrolled into the GO-TARGET-01 study (REC number- 11/SC/0014). These cell populations will then undergo single cell RNA sequencing using the latest state-of-the-art 10x Genomics technology. The large-scale sequencing data generated by this study will then be processed using a linux-based bioinformatics platform and further analyses will be carried out in R.

Training Opportunities

Students will be trained in all relevant wet lab procedures including processing of patient samples, FLOW cytometry, single cell RNA sequencing as well as genomic data analyses by experienced members of the lab and the institute. They will also have an opportunity to attend extensive training sessions in programming and data analyses organized by departments within the university. By participating in our weekly lab meeting, students will acquire scientific presentation skills and can contribute towards the publication of scientific papers.

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3. 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 3 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.

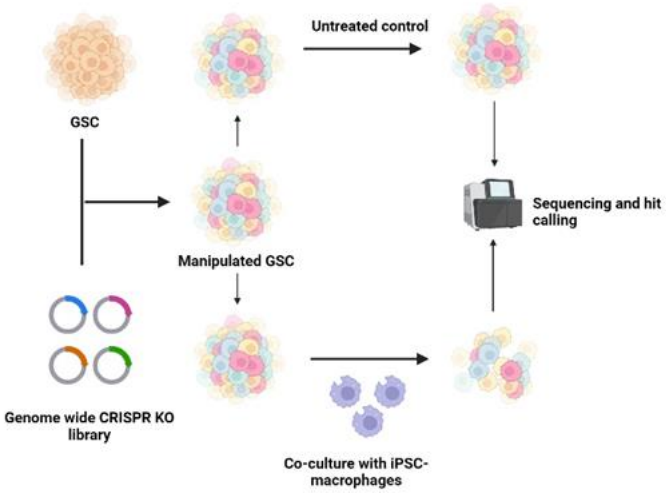

Fig 1: Methodology for CRISPR screening

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.

Rotational Project

Glioblastoma (GBM) poses a significant challenge in oncology due to its aggressive nature and the immunosuppressive tumor microenvironment (TME), where tumor-associated macrophages and microglia (TAMs) play central roles in driving immunosuppression and tumor progression. Despite advancements in immunotherapies, their efficacy in GBM is limited by this immunosuppressive milieu. Immunotherapies targeting "don't eat me" signals like CD47 have shown promise in enhancing macrophage phagocytosis and promoting tumor clearance. However, the dynamics of glioma stem cell (GSC)-macrophage interactions and the tumor-intrinsic features facilitating macrophage-mediated tumor elimination remain poorly understood. This study aims to address this gap by conducting co-culture experiments and CRISPR-mediated genetic screens to elucidate the genetic landscape essential for macrophage-mediated tumor cell clearance. The project involves genetic manipulation of GSCs using a whole-genome CRISPR-Cas9 knockout library, followed by co-culture screening with iPSC-derived macrophages/microglia to investigate key genetic interactions essential for macrophage-mediated phagocytosis. This comprehensive approach will identify hits for further functional validation through cellular assays, representing a critical step in uncovering novel therapeutic targets for GBM.

Training Opportunities

The project offers extensive training opportunities in CRISPR-based cell screening, cell biology, molecular biology, and bioinformatics analysis. Through hands-on experience in gene editing, high-throughput screening, cell culture, and molecular biology techniques, students will develop essential skills in experimental design, data interpretation, literature review, and scientific writing, positioning them for future success in cancer biology and therapeutic research.

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

Primary Supervisor: Olaf Ansorge
Additional Supervisors: Stephen Taylor
Eligibility: Track 3 and Track 4 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.

Rotational Project: Nanopore sequencing for brain tumour diagnostics and research

This six-months rotation will allow the student to gain practical (wet-lab) as well as bioinformatics (theoretical) experience of the application of Oxford Nanopore Technologies (ONT) sequencing for the diagnosis, monitoring and scientific analysis of human brain tumours. Specifically, the student will be given the opportunity to test cutting-edge applications of this technology to human brain tissues; by this we mean those ONT applications that are not yet standard practice such as ONT single-cell sequencing or ONT spatial transcriptomics. For example, the student will apply ONT technology to existing high-quality tumour samples where we hold standard-of-care data such as short-read bulk tissue whole genome sequencing or epigenomic methylation chip data.

This six-months placement would allow for "deep-phenotyping" of a few samples with an emphasis on spatial (microscopic) reconstruction of the data. The focus will be on rare tumour types in order to generate novel pilot data to inform future grant applications (in which a successful student may participate). Examples are fusion-driven neuroectodermal and mesenchymal tumours such as astroblastoma, MN1-altered or mesenchymal chondrosarcoma, HEY1::NCOA2-fused. Even one case done well with the described technologies may result in a publication for the student.

Training opportunities

The placement will allow the student to gain insight into the challenges of applying cutting-edge technologies to human tissues; they would learn wet-lab and bioinformatics principles of ONT sequencing as well as approaches for spatially-resolved digital data integration, analysis and visualisation (e.g. linking a biopsy site visualised in a 3D reconstructed MRI to microscopic and molecular data of the sample).

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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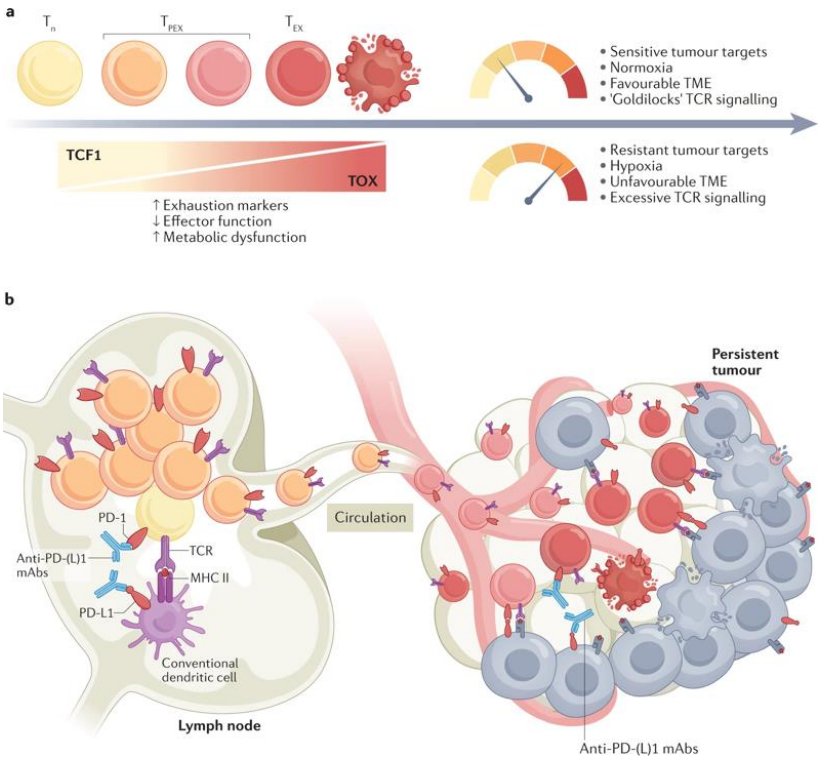
5. An interdisciplinary approach to understanding the quantifying the impact of T Cell exhaustion in cancer – Ruth Baker

Primary Supervisor: Ruth Baker
Additional Supervisors: Helen Byrne and Marco Fritzsche
Eligibility: Track 4 applicants are eligible to apply for this project

Abstract
T cell exhaustion arises in response to prolonged stimulation and over activation, with T cells gradually becoming dysfunctional as they differentiate, and eventually unable to eradicate the antigen responsible for over exposure. In particular, exhausted T cells in the tumour microenvironment undergo a series of changes that leave them unable to eliminate the cancer. Restoring the efficacy of exhausted T cells therefore represents a promising immunotherapeutic strategy, however key questions remain. In particular, a better understanding of how the tumour microenvironment impacts exhaustion will be crucial in the development of new immunotherapies. This project aims to use a multidisciplinary approach to explore this question and, in particular, to investigate how the composition and dynamics of the tumour microenvironment impact T cell exhaustion. It will combine mathematical modelling with experimental data and image analysis techniques to quantify the process of T cell exhaustion, to identify signatures of exhaustion from imaging data, and to understand the importance of spatio-temporal heterogeneities in T cell exhaustion across the tumour microenvironment in predicting tumour growth.

Research objectives and proposed outcomes

Background: Immuno-oncology focuses on harnessing the immune system to act selectively against tumour cells through the production of sustainable T cell responses, thereby lowering the toxicity associated with traditional cancer treatments. Despite many successes, incomplete understanding of the pathophysiology of cancer and the complexity of the immune response, pose significant barriers to progress in developing new therapeutics and designing optimal drug dosing regimen. In particular, the impact of T cell exhaustion, the process whereby T cells differentiate along a spectrum of exhaustion states that have progressively declining functional and proliferative capacity at rates depending on their local microenvironment, is not well understood. Recent *in vitro* experiments carried out in the Fritzsche Lab provide high spatial and temporal resolution imaging data that capture interactions between T cells and tumour cells, as well as T cell



"killing events." As such, they provide an unrivalled opportunity to characterise the process of T cell exhaustion.

Aims and objectives: The aim of the project is to develop mathematical models to analyse, interpret and integrate dynamic imaging data from *in vitro* experiments to better characterise the process of T cell exhaustion. Repeated rounds of model-driven hypothesis generation and experimental validation will provide new understanding of how the rate of T cell exhaustion depends on interactions with tumour and stromal cells. A key challenge in developing and validating the models is that cell-based measurements of exhaustion are not generally available, and so the project will also develop simplified models that represent "average" exhaustion levels on a population-scale and explore the extent to which they can predict observed data. In the longer-term, the development of mathematical models that can predict spatio-temporal patterns of exhaustion will enable testing of new therapeutic strategies that utilise re-programming of cells in intermediate exhaustion states.

The project objectives are:

1. To analyse existing, high-resolution spatio-temporal data collected in the Fritzsche Lab to quantify how T cell killing rates depend on the history of microenvironmental interactions (e.g. number of previous contacts with tumour cells) under different experimental conditions, and to characterise how exhaustion levels evolve in time and space.
2. To develop an agent-based model of T cell exhaustion and T cell-driven tumour cell killing that incorporates "exhaustion state" as an internal variable, and to test and validate this model using the data analysed in Objective 1.
3. To develop a coarse-grained differential equation-based model of the agent-based model developed in Objective 2 that describes how phenotype evolves in time and space, and explore model predictions using a combination of analytical approaches and numerical simulation.
4. To use machine learning-based approaches to obtain simplified models of T cell dynamics in terms of the average exhaustion state of the population, and compare model predictions with those of the differential equation-based model developed in Objective 3.
5. To use the simplified model to assess the impact of different immunotherapeutic approaches, and predict optimised dosing regimen using optimal control theory.

Collaborations: The project will initiate a new collaboration between Baker, Byrne and Fritzsche. The student will make frequent visits to the Fritzsche Lab where they will interact with lab members investigating immuno-oncology. This will enable the student to learn the relevant biology, experimental techniques, and data analysis methods, and to contribute to experimental design. The team will meet with the student on a weekly basis.

Translation potential: This project will use mathematical modelling to provide new insights into how T cell exhaustion impacts tumour growth by interrogating large-scale data sets. It will generate new methods for analysing high-resolution imaging data and strengthen expertise in multidisciplinary approaches to tackling cancer. It will contribute to the scientific themes "Cancer Big Data" and "Immuno-Oncology".

Training opportunities: The student will receive training in mathematical modelling using differential equations and cell-based models, as well as computational Bayesian statistics, optimal control theory, image analysis, experimental design, and multidisciplinary research. Within the Mathematical Institute, the student will be part of the Wolfson Centre for Mathematical Biology, where they will take part in weekly mathematical oncology focus meetings and research skills training sessions, and attend formal weekly seminars. The student will also spend time in the Fritzsche Lab, attending group meetings and relevant seminars at the Ludwig Institute for Cancer Research. There may also be the opportunity for the student to undertake experiments to test and validate their models.

Rotational Project

During the short project, the student will develop a suite of mathematical models of T cell exhaustion building on experimental observations collected in the Fritzsche that characterise T cell – tumour cell interactions. In particular, they will

1. Track T cell – tumour cell interactions from large-scale imaging datasets and use these data to characterise how T cell behaviours change over time as they interact with tumour cells in their local microenvironment.
2. Develop an agent-based model of T cell dynamics, that includes an internal "exhaustion state", and coarse-grain the model to obtain a stage-structured model of T cell dynamics.
3. Explore the extent to which it is possible to validate the models using experimental data, and whether a simpler model, that considers only an population-averaged exhaustion variable, can capture the dynamics of the more complex models.

Training opportunities: Techniques that the student will have the opportunity to develop as part of the rotation project include:

1. Image analysis, cell tracking and event detection (cell-cell interactions).
2. Development of agent-based and differential equation models, use of coarse-graining approaches, computational approaches to simulate models.
3. Calibration of models to experimental data (including parameter estimation techniques), and model validation.

Ideal student background: The student will have an undergraduate degree in mathematics, physics or engineering. They should ideally be familiar with mathematical modelling and scientific computing techniques, and have experience in applying these methods to problems in biology.

References

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

Primary Supervisor: Claire Bankhead
Additional Supervisors: Brian Nicholson
Eligibility: Track 3 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.

Rotational Project: Understanding unwarranted variation in cancer testing in primary care

Primary care is the first point of contact for most people who experience symptoms and are diagnosed with cancer. Sociodemographic variation in factors that may influence cancer outcomes have been observed at numerous points along the diagnostic pathway. The candidate will analyse contemporaneous electronic health records data from primary care to identify patient groups who undergo less testing for cancer symptoms than expected. The will then develop a plan with patient and public representatives and relevant communities to further understand and address any variation deemed unwarranted.

The candidate will develop an understanding of epidemiological research, particularly using large routinely collected healthcare data, and qualitative research methods to develop evidence-based guidance to inform policy and practice.

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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7. 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 3 and Track 4 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.

Rotational Project: Developing high-throughput AI-based methods for detecting tumour-associated changes in tissue architecture from imaging

Despite the success of immunotherapy in many cancers, there are still many cancers with very poor survival rates, including pancreatic ductal adenocarcinoma (PDAC) (<7% 5-year survival), glioblastoma (<7% 5-year survival) and renal cell carcinoma (15% 5-year survival for stage 4). All of these cancer types pose formidable challenges, and have not benefited from immunotherapies, which have revolutionised the treatment of many other malignancies. We, and others, have shown each tumour, each immune system and each patient is different Current treatment options lack personalisation to leading to suboptimal outcomes, with insufficient predictive tools for guiding clinicians to the best treatment option for patients, and barriers for adoption of translation of novel approaches. **We aim to tackle all these issues through tissue avatar systems, in which tumour tissue from a patient is thinly sliced and cultured, such that each section can generate a model to test different treatments outside the patient's body, to determine optimal treatment strategy for that patient, and trialling novel therapeutic approaches**. Given the complexity of the tumour microenvironment and the limited effect of current therapeutic approaches, tumour avatars are well placed for driving step changes in improving patient care in a reliable, reproducible and cost-efficient manner.

A fundamental basis for this approach is to quantify how the patient tumour behaves differently between different conditions. These changes may be detected quickly and cheaply via imaging approaches (step 2 in *Figure 1*), however, there are no robust computational methods for taking imaging data to extract representative features of the tumour microenvironment that will describe how each drug/condition is impacting the tumour. Secondly, the data size and management from large imaging sections poses a significant challenge and cost. **This project will contribute to the development of real-time and scalable development of tumour avatars through bypassing computationally expensive processing steps.**

We aim to:

1. Capture and quantify changes in cellular composition, interactions and acellular components within the tumour, allowing for comparison to other images from the same tumour under different conditions and between patient tumours.
2. Develop improved approaches for compressing imaging data throughout the processing pipeline and archiving to ensure cost-effectiveness.

This proposal outlines a comprehensive strategy to establish this cutting-edge platform for precision oncology by addressing imaging-data extraction problems using cutting-edge machine learning (ML) and artificial intelligence (AI) based on our established imaging approaches. This grant will provide the support for the development of a suite of tools for tissue image analysis and image storage that will be of broader interest to the research community.

Ultimately, our goal is to create a tool that can forecast patient responses to specific treatments, and this will be a key component in this aim. This will help us take a big step towards a more effective, responsible and personalised approach to pancreatic cancer care.

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.

References:

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

Primary Supervisor: Hagan Bayley
Additional Supervisors: Ahmed Ahmed
Eligibility: Track 3 and Track 4 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.

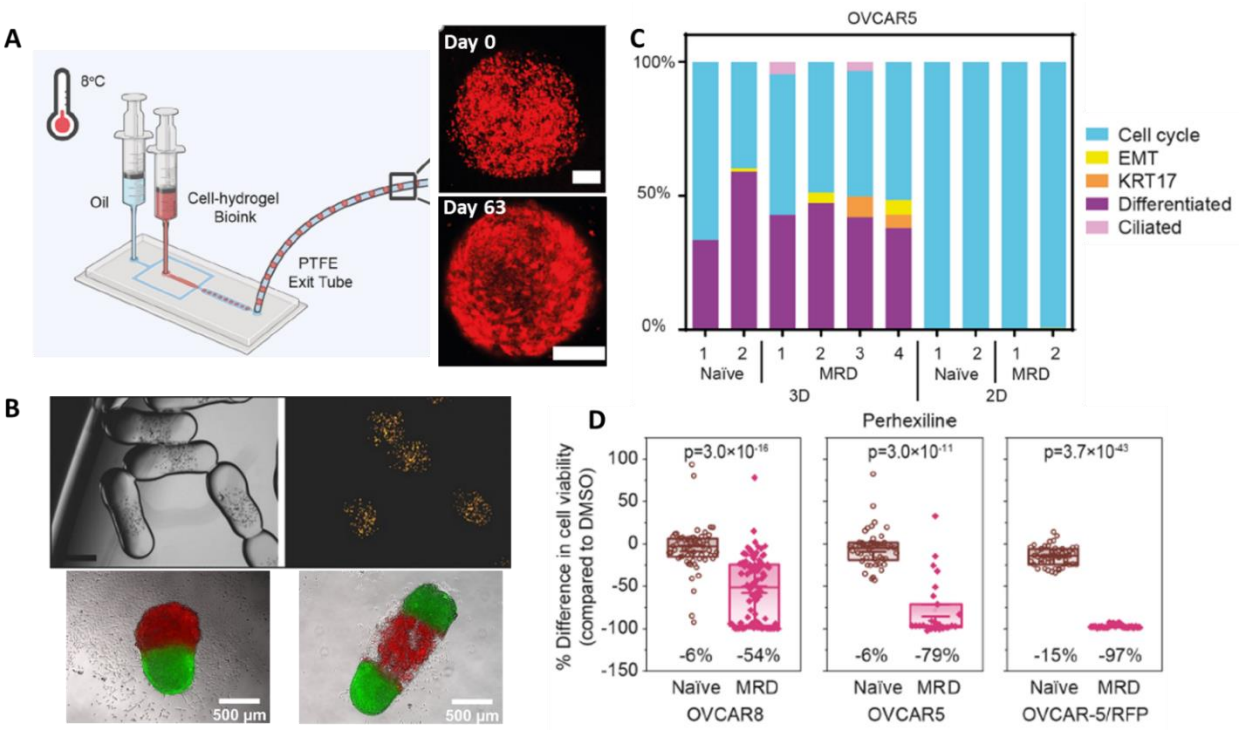

Figure 1: Microfluidic-based 3D microtumour technique and drug testing using the 3D MRD microtumours.[6] **A**, Schematic illustration of 3D microtumour fabrication by the microfluidic platform (left) and long-term culture of 3D microtumours composed of OVCAR-5/RFP (red) and Matrigel (right). Scale bars 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.

Rotational Project: Microfluidics-based 3D Microtumours to Model Ovarian Cancer Minimal Residual Disease

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 use microfluidics to fabricate 3D MRD microtumour model using cancer cells derived from patients. We will test the molecular characteristics of the MRD microtumour model and the influence of different extracellular matrix materials on the MRD cells.

Training Opportunities

The potential DPhil student will be trained in the following fields: 1) Establishment and maintenance of cancer organoids (derived from patients' biopsies) and drug treatment to generate the MRD cells; 2) Microfluidic fabrication and hydrogel manipulation; 3) Microscopy (especially confocal microscopy) and general molecular biology techniques.

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 bio fabrication 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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9. The project has been removed and is no longer available for selection

10. Understanding the origin of metaplasia – Francesco Boccellato

Primary Supervisor: Francesco Boccellato
Additional Supervisors: Jan Bornschein
Eligibility: Track 3 and Track 4 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.

Rotational Project: Generation of mucosoids from intestinal metaplasia

Intestinal metaplasia of the stomach is characterized by the presence of epithelial cells in the stomach exhibiting features of the intestine. Patients with intestinal metaplasia are at a higher risk of developing gastric cancer. This lesion typically arises as a consequence of chronic infection with *Helicobacter pylori*, a gram-negative bacterium linked to the onset of gastric adenocarcinoma. A key characteristic of metaplastic cells is their expression of intestinal-type mucus. The project aims to demonstrate that intestinal metaplasia develops as a response to reduce infection burden. Specifically, we hypothesize that the mucus produced by metaplastic cells possesses enhanced bactericidal properties compared to that of the gastric mucosa. To test this hypothesis, the student will isolate stem cells from biopsies of intestinal metaplasia and cultivate them into mucosoid cultures. Stomach mucosoids are stem cell-driven models of epithelial cells that mimic the gastric mucosa at homeostasis, including the secretion of acid, digestive enzymes, and mucus. By generating mucosoids from intestinal metaplasia, the student will harvest and analyse the mucus, comparing its properties with those from healthy gastric samples. This approach aims to provide insights into metaplasia as a protective tissue mechanism against infections

Training opportunities

Jan Bornschein, the clinical supervisor of this project at the John Radcliffe Hospital, is responsible for identifying patients with intestinal metaplasia and providing biopsies for this and other studies. The student involved in this project will learn to isolate epithelial cells from these biopsies and cultivate them initially as organoids and subsequently as mucosoids. The generation of organoids aims to increase the initial cell numbers to reach the minimal density required for mucosoid cultivation. The primary goal of cultivating mucosoids is to analyze the properties of mucus produced by metaplastic cells. This mucus will be harvested, incubated with H. pylori, and tested for bactericidal properties by replating the bacteria and counting the colony-forming units. To investigate the reasons behind the bactericidal properties, the project will focus on detecting human bactericidal proteins, known as antimicrobial peptides (AMPs), which are essential for controlling bacterial gut homeostasis. AMPs will be detected by immunofluorescence and confocal microscopy on the mucosoids and by western blot analysis of the secreted mucus. The expression of these antimicrobials will also be validated in human tissue using formalin-fixed, paraffin-embedded (FFPE) sections from the same biopsies used to generate the mucosoids.

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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11. 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 3 and Track 4 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.

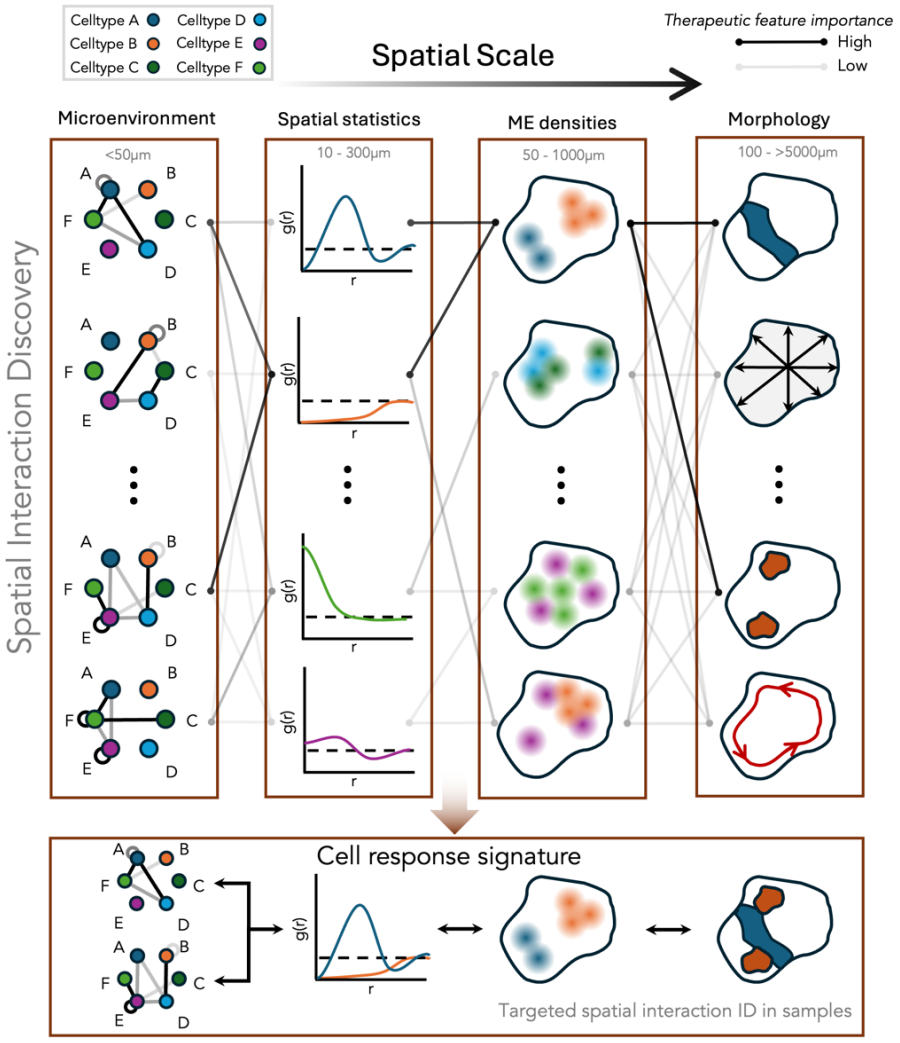

Figure 1. The development and application of multiscale spatial cellular response signatures for therapeutic quantification. Spatial features of treated and untreated samples span spatial scales from cellular microenvironments (ME) to global morphological properties and will be organised using a spatial 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

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.

Rotational Project

In a shorter rotation project, track 4 candidates will work within a multidisciplinary team of mathematicians and biologists to learn the fundamentals of spatial biology image generation, cell segregation, and advanced mathematical analysis and interpretation. Students will be supported to learn how to use our in-house software for spatial analysis. They will then apply these skills to characterise and quantify the key dynamic changes in existing multiplex spatial biology data sets from pre and post treatment mouse cancer models and, in so doing, identify a smaller set of discriminatory cell markers that distinguish pre- and post-treatment tissues. They will then test the predictive power of this subset of cell markers on unseen imaging data.

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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12. 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 3 and Track 4 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.

Rotational Project: Manufacturing and Characterisation of Ultrasound-responsive Microbubbles for the Delivery of Chemotherapy Drugs for Bone Cancer

Gas microbubbles are spherical particles comprising a gas core surrounded by a stabilising shell. They are routinely used clinically as contrast agents in ultrasound imaging because of their ultrasound responsiveness, which is provided by their ability to undergo a repeated oscillatory motion upon exposure to ultrasound pressure waves. Microbubbles have also been researched for applications requiring localised and enhanced delivery of therapeutic compounds, as microbubble oscillations can transiently permeabilise biological barriers to facilitate drug penetration. Despite the extensive research being conducted in this area, only a very limited body of work has evaluated the application of ultrasound-responsive agents as a drug delivery modality in bone disease. This project will specifically evaluate their potential as a treatment modality against bone cancer. The primary aim of this rotation project is to establish and characterise candidate formulations of drug-loaded microbubbles (dMB) that could be employed as a delivery system for active pharmaceutical ingredients for the treatment of bone metastasis. The specific objectives include:

(1) To manufacture anti-cancer drug-loaded microbubbles (months 1-3). Combinations of established anti-cancer drugs and clinically-relevant microbubble formulations will be assessed for their potential to generate a dMB construct. Microbubble formulations will comprise a phospholipid shell and a core made of an heavy gas. Different drug loading approaches will be evaluated, including surface attachment and intercalation within the microbubble shell, depending on the drug's chemistry. Manufacturing methods used will include mechanical agitation and microfluidic-based ones, depending on the physico-chemical properties (i.e. temperature sensitivity) of the drug candidate. Formulations will be assessed based on their stability (quantified as microbubble size and concentration over time) and drug loading capacity.

(2) To characterise the ultrasound responsiveness of dMBs (months 3-4). Shortlisted formulations of dMBs (from Objective 1) will be assessed for their ability to respond to ultrasound stimulation. This will be quantified using both clinical B-mode imaging as well as passive cavitation detection methods. Moreover, drug release both in the absence of ultrasound and upond ultrasound activation will be quantified. Ultrasound exposure parameters varied will include frequency, duty cycle, acoustic pressure, and treatment time. The potential for applying repeated cycles of stimulation (and concurrent drug release) will also be evaluated.

(3) To assess microbubble behaviour within a microfluidic-based bone model (month 5). Optimised ultrasound parameters (from Objective 2) will be evaluated for the ability of inducing dMBs oscillation and controlled drug release in a miniaturised bone model, replicating the physical (including acoustical) properties of bone. This model has already been developed in an ongoing research project, evaluating the use of ultrasound-responsive particles for bone fracture repair.

As part of this rotation project, the PhD candidate will undergo a number of training opportunities including in microbubble fabrication (agitation or microfluidic-based techniques) and chemical formulation (thin-film hydration), microbubble characterisation (particle sizing instrumentation and microscopy), therapeutic ultrasound instrumentation (including clinical ultrasound systems), and microfluidic-based equipment. General research skills, including design of experiment, data/statistical analysis, and research communication will be further developed through discussion in regular group and individual meetings, and presentations at Departmental meetings.

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

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13. 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 & Tim Elliott
Eligibility: Track 3 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

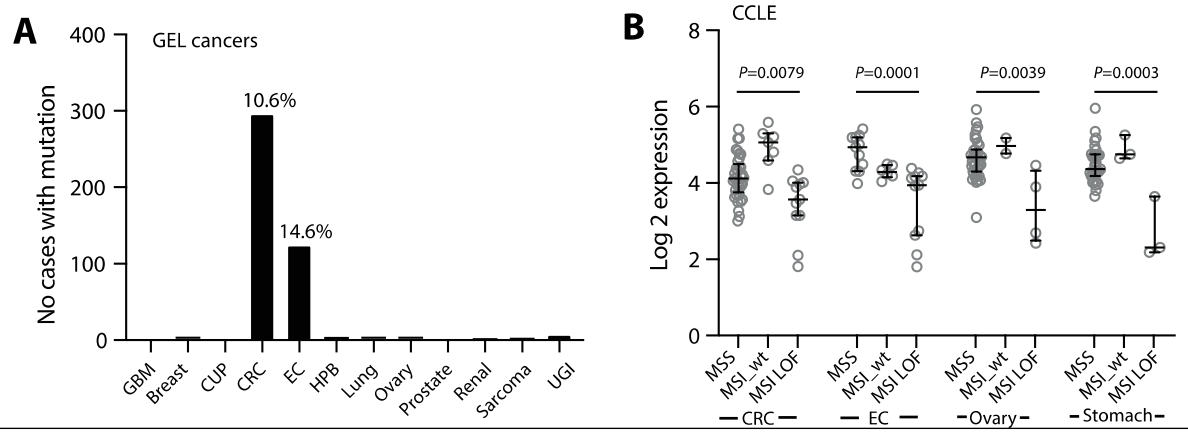

Figure 1. (A) Frequency of novel LOF immune escape mutation in Genomics England cancers by tumour type. (B) Association of MSI and immune escape gene LOF mutation status with expression in Cancer Cell Line Encyclopedia (CCLE)

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.

Rotational Project

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. However, the acquisition of immune escape mutations enables cancers to elude immune destruction, and become resistant to immunotherapy. Our unpublished analysis of cancers from the Genomics England 100,000 Genomes Project (100KGP) has identified two novel candidate immune escape mutations which occur commonly in dMMR cancers. Both act within the antigen processing and presentation pathways, providing a strong rationale for their further investigation. During this rotation, the student will analyse multi-omic data (genomic/transcriptomic/spatial/immunopeptidomic) from colorectal and endometrial cancers and precancers generated from the Genomics England and LynchVax cohorts to delineate the frequency and correlates/putative consequences of the novel immune escape mutations. Depending on experience, the student may be able to undertake preliminary functional characterisation of these mutations**.**

Training opportunities

Analysis of genomic, transcriptomic, spatial and immunopeptidomic data.

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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14. Quantification of circulating p53 antibodies to predict cancer development in high risk individuals – Jason Davis

Primary Supervisor: Jason Davis
Additional Supervisors: Matthew Bottomley
Eligibility: Track 3 and Track 4 applicants are eligible to apply for this project

Abstract
Cancer is a leading cause of death and illness in patients receiving pharmacological immunosuppression, such as after kidney transplant. The most common cancer, cutaneous squamous cell carcinoma (CSCC), is over 100 times more common compared to the general population. We previously undertook a cohort study of long-term kidney transplant recipients (KTR) at high risk of developing cancer and have demonstrated that perturbations in the circulating immune system may identify those at increased risk of subsequent cancer [1, 2]. However, these are all cell-based markers which have limitations in the they require specialised sample processing and flow cytometric analysis. A serum-based marker would be preferable in processing of these samples are quicker (ergo cheaper) and more standardised across laboratories.

An early event in CSCC development is mutation of the tumour suppressor gene *TP53* [3]. This may allow for the development of an immune response against the abnormal p53 protein, including the development of p53 autoantibodies (p53aa). Recently, a sophisticated and ultrasensitive sensor was developed by the research group of the primary supervisor for the selective detection of p53aa against a rarely mutated part of the protein [4]. The sensory configuration combines state of the art magnetically assisted microfluidics, capture nanoparticles and amplified electrochemistry.

This DPhil is an entirely new collaboration between the Nuffield Department of Medicine and the Department of Chemistry. The project will combine the expertise of the two laboratories by quantifying circulating p53aa levels in previously collected sera from high-risk kidney transplant recipients and non-immunosuppressed controls with and without history of previous cancer. The study will evaluate whether prior cancer is associated with altered p53aa levels, as well as evaluating their predictive value for subsequent cancer development based on previously collected outcomes data for the cohort.

The findings from this DPhil may translate into a novel method to identify patients at high risk of developing CSCC, which may allow for pre-emptive intervention to reduce future cancer burden.

Research objectives and proposed outcomes
This project has academic objectives from a chemistry (analytical) and a medical (clinical) perspective.

Analytical perspective:

- Build upon the use of synthesised magnetic immunonanoparticles and advanced microfluidics to selectively extract p53aa from low volumes of serum, using highly non-fouling (likely polymeric) surface chemistry on the nanoparticles and the integration of p53aa-specific peptide antigen mimics.
- Refine sensor performance, including:
    - Exploration of the integration of new signal-generating modalities, both within the capture particles and at the sensor interface.
    - Refinement of the microfluidics target isolation configuration.

Clinical perspective:

- Confirm the presence of p53aa antibodies within sera from KTR and non-immunosuppressed controls – and then evaluate (a) their association with prior history of CSCC/other cancer and (b) predictive value for subsequent CSCC/cancer development.
- Depending on the candidate's interest and initial findings, further work within the project may include:

    - Evaluation of circulating B cell populations in patients exhibiting p53aa;
    - Delineation of the isotype of detected antibodies
    - Detection of these antibodies within excised CSCC from these patients, potentially with spatial transcriptomic analysis to evaluate the local effect of the presence of these antibodies.

This award would also cement a new collaboration between the COI and Department of Chemistry.

Translational potential

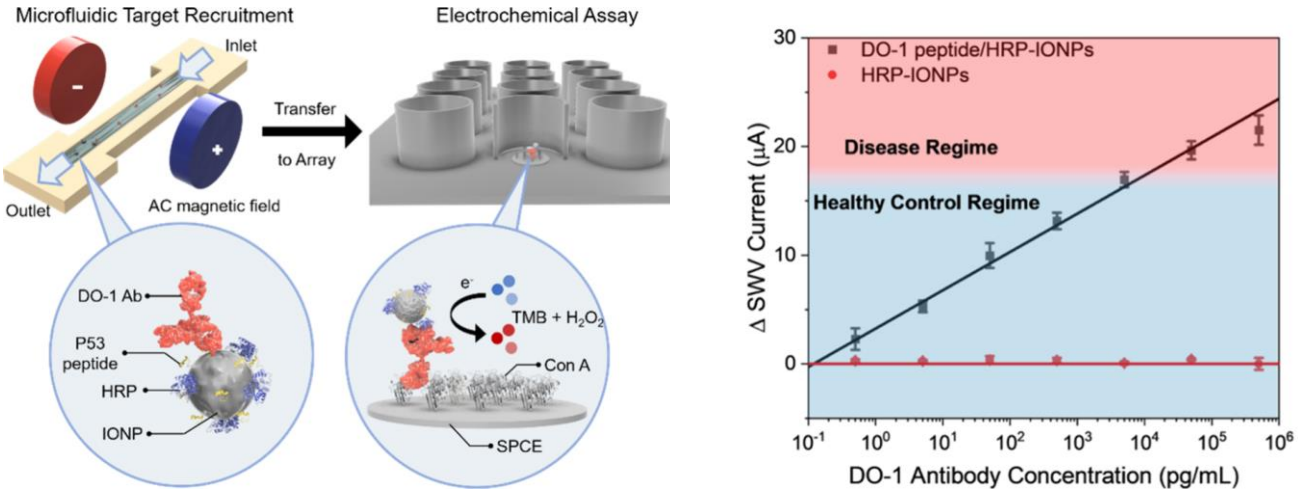
This represents a truly translational project, with supervision from both a chemist and a practicing clinician scientist. Cancer is a major source of morbidity and mortality in immunosuppressed populations. Whilst various interventions have been proposed to CSCC risk in KTR, accurate stratification of cancer risk to allow targeted deployment of these are an unmet need [5].

This project straddles chemistry and clinical practice. The candidate will further the development of a cutting-edge and novel approach to detect p53aa, combined with evaluation of its clinical performance as a predictive biomarker. The development of new signal generating technologies, new assaying capabilities and nanoparticle chemistries has applicability to biomarker assays generally, with potential for use across the clinical spectrum.

Training opportunities

The student will gain understanding and experience in the development of novel assays for clinical use, including their validation and testing on patient samples. They will also gain insight into cancer immunology and the use of biomarkers to predict cancer risk.

Within the Davis Group, they will have extensive training in electroanalysis, assay development, microfluidics, 3D printing and nanoparticle chemistry, all strongly supported by the infrastructure and personnel in the Davis Group. Through training in the Bottomley Group, they will gain experience in handling human samples, such as serum. They will receive training in univariate and multivariate survival analysis, including use of competing risk models. Depending on their interest, they would also have the option to receive training in undertaking and analysing immuno-histological and transcriptomic approaches such as immunofluorescence and spatial transcriptomic analysis of excised tumours.



Left: Schematic depiction of the methodologies to be utilised in capturing p53aa's using magnetic (IONP) nanoparticles from serum in a microfluidic chamber prior to electrochemical assays at (Con A) antibody interacting screen printed carbon electrodes (SPCEs). TMB is a HRP enzyme substrate used to generate an amplified signal. Right: Preliminary data demonstrating the ability of optimised electroanalytical assays (SWV=square wave voltammetry) to quantify p53aa levels across a broad and clinically relevant concentration range. Control sample analysis (without p53aa capture) shown in red.

Rotational Project: Comparative Evaluation p53 immunoassays

Among the myriad of cancer biomarkers, p53, encoded by the TP53 gene has gained prominence due to its core antiproliferative function in preserving genomic stability. In more than 50% of human cancers, aberrant p53 proteins, encoded by a mutated TP53, accumulate in cancer cells and may further promote tumour growth and metastasis. This accumulation manifests as an increased concentration of p53 proteins in serum and has, for example, been assayed at levels markedly higher than those of healthy controls in patients. The robust assaying of circulating p53 is, however, made challenging due to both the heterogeneity of both its mutated forms and post translational modifications. The abnormal accumulation of p53 proteins triggers the generation on anti-p53 antibodies. These antibodies are largely structurally consistent, and their quantification, at levels (~100ng/mL), i.e spiking to hundreds of times higher than that of the antigen in serum, is more accessible.

This 6-month project will develop comparative p53 autoantibody assays by both novel electrochemical methods and comparative 'gold standard' optical/ELISA methods. After initial training, analyses will be carried out on ~20 KTR and control patient serum samples. The project will establish a firm grounding in basic immunoassay methods, correlating data sets and an initial view of patient samples data sets prior to developing more sophisticated antibody pre-capture and amplified assaying methods.

Training opportunities

- Training in basic electrochemistry/electro analysis
- Training in basic surface chemistry
- Training in blood handling
- Training in ELISA based immunoassays
- Training in electrochemical immunoassays
- Training in statistical analysis
- Training in presentational and writing skills

Ideal student background: The ideal student would have an undergraduate degree or Masters in Chemistry, biochemistry or medical sciences. It would be beneficial, though not mandatory, if the student had prior exposure to analytical assays and/ or handling of human samples.

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15. Equitably implementing improvements in cancer detection – Anna Dowrick

Primary Supervisor: Anna Dowrick
Additional Supervisors: Brian Nicholson
Eligibility: Track 3 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.

Rotational Project: Understanding barriers to the equitable implementation of a novel early detection technology

It is important to actively plan how innovations in cancer detection will help to address inequality in cancer outcomes, and to consider how both the data used and the process of implementing change can deliver this. The candidate will conduct secondary analysis of existing qualitative data collected from patients and healthcare professionals during past or ongoing implementation projects of early detection technologies to explore the equity implications of early cancer detection initiatives. This will build on evidence synthesis and patient and public engagement to understand the needs of selected minoritized communities.

Training opportunities during this rotation the candidate will develop an understanding of qualitative evidence synthesis, grounded theory, framework analysis, academic writing, and writing for a lay audience.

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

Primary Supervisor: Claire Edwards
Additional Supervisors: Karl Morten
Eligibility: Track 3 and Track 4 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

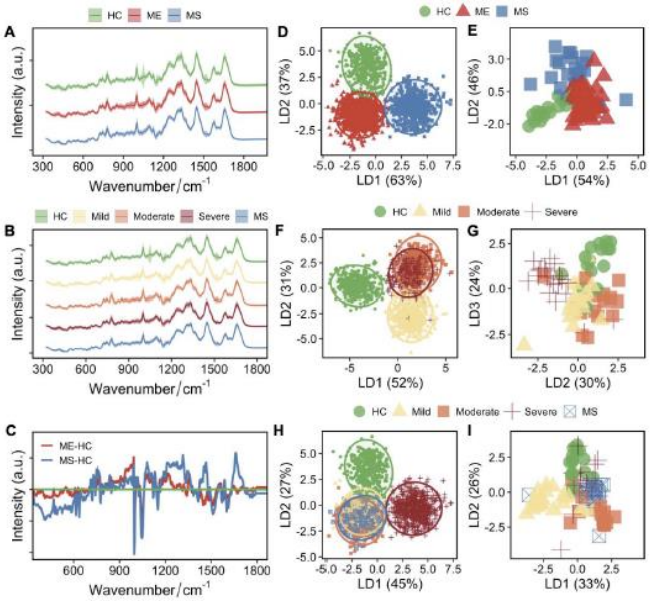

FIGURE 1. SINGLE-CELL RAMAN FINGERPRINTS FOR MYALGIC ENCEPHALOMYELITIS/CHRONIC FATIGUE SYNDROME (7).

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 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.

Rotational Project

This project 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. The rotation project will investigate the effect of novel mitochondrial metabolism disruptors in prostate cancer cells and will use Raman microscopy to compare metabolic profiles of paired bone metastatic and non-metastatic prostate cancer cell lines.

Training Opportunities

The 6-month rotation project will be designed to expose the student to many of the experimental techniques that will be used during the DPhil project. Cellular and molecular techniques will be used to study the effect of pharmacological mitochondrial targeting on prostate cancer biology, including growth, cell death, metabolism, migration, dormancy and senescence. These experiments will be performed alone, and in coculture with bone cells. Techniques will include protein profiling, RNA-Seq, flow cytometry and metabolic analysis. Training will be provided in single-cell Raman microscopy, using paired cell lines that differ in their bone metastatic ability. This will include microscopy, metabolic profiling, bioinformatic analysis and machine-learning. In addition, students will benefit from exposure to a range of techniques by the Edwards and Morten groups, including circulating tumour cell isolation and preclinical models of bone metastasis, in order to give them a thorough insight into the DPhil project

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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17. 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 3 and Track 4 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.

Rotational Project

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. You will validate these results in an independent cohort of samples, and localise T cell subsets, along with other stromal and immune subsets in the tumour microenvironment using multiplexed immunohistochemistry of OAC samples banked for the COMBATcancer project.

Training opportunities Spectral flow cytometry and multiplex immunohistochemistry of human patient samples. Quantitative biology including fundamental bioinformatic and data analytics.

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

Primary Supervisor: Fumiko Esashi
Additional Supervisors: Bass Hassan
Eligibility: Track 3 and Track 4 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 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

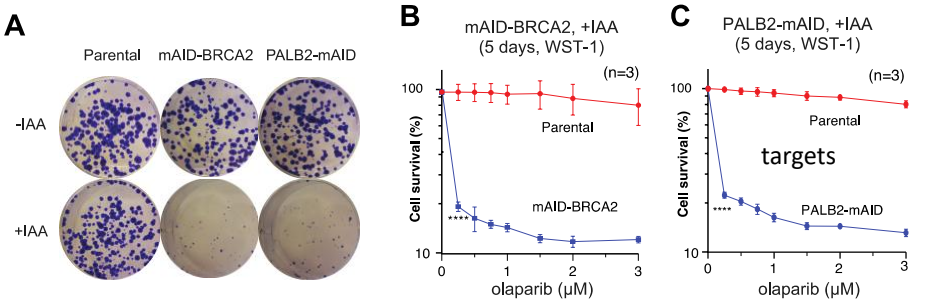

Figure 1. A. HCT116 mAID-BRCAs/PALB2-mAID cells were seeded in 6-well plates, and grown for 10 days with and without auxin (IAA). Colonies were then fixed and stained with crystal violet. **B-C:** HCT116 mAID-BRCA2/PALB2-mAID and parental HCT116 cells were first treated with IAA for 2 hours, and subsequently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

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 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.

Rotational Project: Understanding BRCA2 and Cancer Risk

The breast cancer susceptibility gene 2 (*BRCA2*) is crucial for maintaining genomic stability. Mutations in the *BRCA2* gene are strongly associated with an increased risk of developing several types of cancers, most notably breast and ovarian cancer. This condition is often referred to as Hereditary Breast and Ovarian Cancer syndrome (HBOC). Individuals with inherited mutations in *BRCA2* have a significantly higher likelihood of developing these cancers compared to the general population. Interestingly, while *BRCA2* mutations are linked to cancer, complete loss of BRCA2 function in normal cells typically results in cell death. This paradox suggests that cancer cells which are deficient in *BRCA2* must acquire additional genetic changes that allow them to survive and proliferate despite the absence of this critical gene.

We hypothesis that *BRCA2*-deficient cancer cells survive due to the loss or gain of other genetic factors that compensate for the absence of BRCA2. These compensatory genetic alterations help the cancer cells maintain essential functions, such as DNA repair, that would otherwise be compromised. To identify these compensatory genetic factors, we have conducted a genome-wide screen. This approach aims to find genes that, when inactivated, are lethal to cells lacking BRCA2. This concept, known as synthetic lethality, implies that while cells can survive the loss of either BRCA2 or **another specific gene** independently, the simultaneous loss of both genes results in cell death. By identifying such genes, we hope to uncover potential targets for cancer therapy, particularly for cancers associated with BRCA2 mutations.

Training opportunities

During the six-month rotation, the student will be involved in evaluating the candidate factors identified from the genome-wide screen. This evaluation will focus on understanding how these factors contribute to genome stability in the absence of BRCA2. The student will employ various techniques commonly used in the group, which may include:

Molecular Biology Techniques: These might include PCR, Western blotting, and cloning to analyse the expression and function of candidate genes.

Cell Biology Techniques: Techniques such as cell culture, transfection, and RNA interference (RNAi) to manipulate gene expression in BRCA2-deficient cells.

Genomic and Bioinformatics Analyses: Use of bioinformatics tools to identify and analyse genetic interactions.

Functional Assays: Assays to assess cell viability, DNA repair efficiency, and genomic instability in cells with modified expression of candidate genes.

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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19. The development of inhibitors of DHCR24 to validate its role in clear cell Renal Cell Carcinoma – Matthew Fuchter

Primary Supervisor: Matthew Fuchter
Additional Supervisors: Ester Hammond
Eligibility: Track 3 applicants are eligible to apply for this project

Abstract
Clear cell Renal Cell Carcinomas (ccRCC) are an aggressive subtype of kidney cancer that have limited therapeutic options. These tumours are distinct and histologically defined by the presence of large lipid stores called lipid droplets (hence the name "clear cell") meaning they display an altered metabolism that define their clinical presentation. Lipid droplets store free fatty acids and cholesteryl esters, which are predominantly synthesized by *de novo* lipogenesis and the sterol synthesis pathway, respectively. This altered metabolism in ccRCCs is genetically underpinned by loss of the tumour suppressor VHL and stabilisation of the Hypoxia Inducible Factors (HIFs). HIFs are known to drive dependency on lipogenic enzymes, but little is known about their impact on sterol biosynthesis machinery [2]. We have identified cholesterol biosynthesis proteins to be important for ccRCC growth, including the terminal enzyme 24-dehydrocholesterol reductase (DHCR24) [1]. DHCR24 acts at the terminal end of the sterol biosynthesis (Figure 1), where it takes Bloch pathway sterols and reduces them to generate KR pathway sterols, ultimately generating cholesterol. Utilising siRNA to deplete over 220 metabolic enzymes, we found that silencing of DHCR24 reduced ccRCC cell number, while having no effect on the normal cell line HK-2. Curiously, silencing of the upper sterol synthesis pathway i.e. HMGCR, had no effect on cell viability, suggesting that the pathway in its entirety is not essential, but only terminal components, such as DHCR24. This is in line with previous studies that have shown that ccRCC cells replete in lipid droplets are auxotrophic for cholesterol, meaning that they do not synthesize cholesterol from acetyl-CoA but are reliant on sterol uptake [3].

In unpublished work we have developed a preliminary steroidal inhibitor of DHCR24 (called P-3), which we are using to further validate DHCR24 as a target and further understand the consequences of DHCR24 inhibition in ccRCC. We find that our inhibitors of DHCR24 have a selective effect on ccRCC and result in a potentially new form of ferroptosis as the main mechanism of cell death (Figure 1). This project will build on this prior work, aiming to improve our current DHCR24 inhibitors and use them to specifically explore the mechanistic link between DHCR24 dependency and hypoxia.

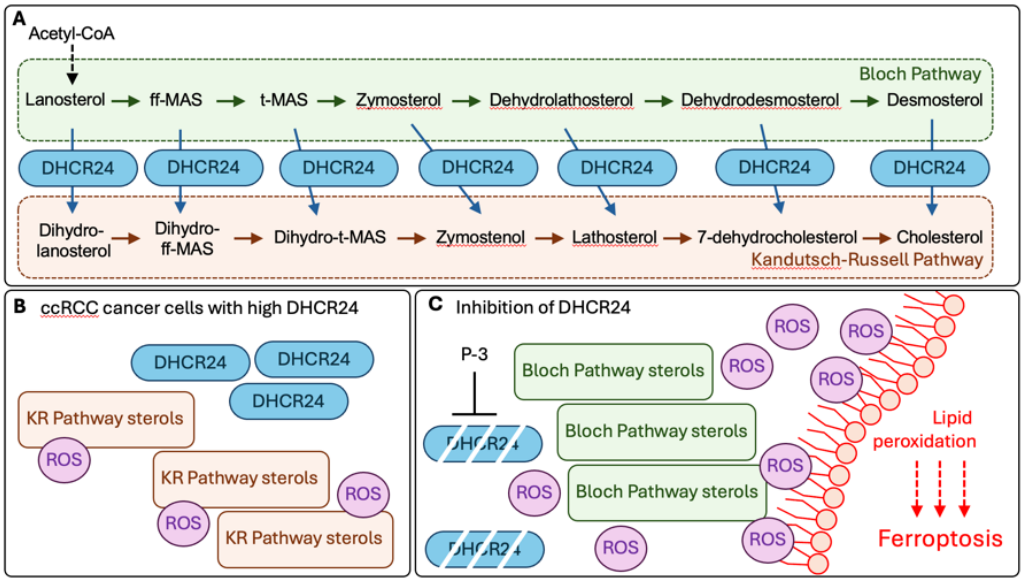

Figure 1: Sterol synthesis in ccRCC cells. A) Shows the Bloch and Kandutsch-Russell (KR) pathways, with DHCR24 being the central enzyme controlling KR synthesized sterols. B) Shows DHCR24 generating ROS-scavenging KR sterols. C) Shows the induction of ferroptosis after DHCR24 inhibition.

Research objectives and proposed outcomes

The rationale for this project is twofold. Firstly, we aim to further optimise our inhibitors of DHCR24, which can then be used to validate the role of this enzyme as a target in ccRCC. Our initial inhibitor (P-3) was developed as a mimic of the natural substrate of DHCR24. While P-3 is already more specific than comparator molecules in the literature (such as Triparanol and SH-42), there is still significant potential to improve the potency of our inhibitors as well as their physiochemical properties to allow for optimal tissue penetration. Secondly, we aim to explore the mechanistic link between inhibition of DHCR24 in ccRCC and hypoxia. cRCCs are the typical hypoxic tumours due to VHL loss and HIF protein stabilization. We have already observed that an increase in DHCR24 protein expression in ccRCC cells correlates with HIF2 expression and we aim to further explore this relationship. Interestingly, it has been shown previously that HMGCR regulation by low oxygen is independent of HIFs [4], meaning that regulation of DHCR24 by HIFs is novel and distinct to other proteins in sterol biosynthesis. Prof. Fuchter is an experienced medicinal chemist who has recently moved to Oxford. Prior to that he was a Professor of Chemistry at Imperial College London and Director of the Imperial College Centre for Drug Discovery Science. Prof. Hammond is an expert in redox and hypoxia biology in cancer. Thus their collaborative partnership and co-supervision will be highly complementary with all the necessary technical expertise for the proposed project. The project will be additionally in collaboration with Dr. Barrie Peck (Barts Cancer Institute). Dr. Peck's main academic interest is in identifying novel fundamental metabolic dependencies in cancer and translating this knowledge into clinical advances. His work previously uncovered the novel role for DHCR24 in ccRCC and he and Prof. Fuchter previously collaborated to identify P-3.

Translational potential of the project.

In the UK over 13,322 cases of renal cancer are diagnosed annually, establishing it as a significant threat to kidney health. The incidence of kidney cancer is also increasing, due to factors such as obesity and poor diet, meaning that this disease burden will grow. ccRCC is the most common subtype of renal cancer (70-80% of cases) and is extremely aggressive. It is a tumour type of significant clinical unmet need due to the lack of efficient standard and targeted therapies. Current clinical trials are focussed on assessing the efficacy of immunotherapies, yet is appreciated that there is need to identify novel, robust, synthetically lethal targets that impact cells that have lost VHL [5]. When we assessed whether sterol biosynthesis is associated with ccRCC aggressiveness, only increased expression of DHCR24 was associated with a poorer overall survival (p=0.0021). Immunotherapy is currently being investigated as a novel therapeutic strategy to treat ccRCC, but this will only be efficacious in patients who have a high neoantigen burden. Importantly, higher DHCR24 expression is still prognostic of a poorer overall survival in neoantigen low tumours (p=0.00062), suggesting that its increased expression could be responsible for driving disease aggressiveness and could be a good anti-cancer target in these patients that will not benefit from immunotherapy. This project will provide novel inhibitors and novel target validation of DHCR24 that should lay the ground work for further translational efforts towards new treatments for ccRCC. Prof. Fuchter has significant experience in translational drug discovery in oncology, being an inventor of two drugs currently in clinical trials and founder of a new immunotherapy spinout company NK:IO Ltd.

Training opportunities

This project represents a new collaborative interaction between Prof. Fuchter and Prof. Hammond. Both supervisors have a strong track record for multidisciplinary cancer science and supervising doctoral and undergraduate students. The student will be based jointly in the Fucher lab (Department of Chemistry) and Hammond lab (Department of Oncology), with strong links to the Peck group. The student will have the

opportunity to learn a wide range of techniques spanning synthetic chemistry, medicinal chemistry and chemical biology, as well as molecular and cell biology techniques (tissue culture, protein analysis, qRT-PCR, use of hypoxia chambers, metabolomics and 3D cultures etc.)

Rotational Project

Clear cell Renal Cell Carcinomas (ccRCC) are an aggressive subtype of kidney cancer that have limited therapeutic options. These tumours are distinct and are histologically defined by the presence of large lipid stores called lipid droplets (hence the name "clear cell") meaning they display an altered metabolism that define their clinical presentation. We have identified cholesterol biosynthesis proteins to be important for ccRCC growth, including the terminal enzyme 24-dehydrocholesterol reductase (DHCR24). Importantly, when ablated in a large panel of renal cell lines, silencing of DHCR24 had no effect on normal cells, suggesting it is a cancer-specific dependency.

In unpublished work we have developed a preliminary steroidal inhibitor of DHCR24 (called P-3), which we are using to further validate DHCR24 as a target and further understand the consequences of DHCR24 inhibition in ccRCC. We find that our inhibitors of DHCR24 have a selective effect on ccRCC and result in (a potentially new form of) ferroptosis as the main mechanism of cell death (Figure 1). This project will build on this prior work, aiming to using synthetic and medicinal chemistry to improve our current DHCR24 inhibitors and conduct preliminary cell-based experiments to explore the mechanistic link between DHCR24 inhibition and hypoxia.

Training Opportunities

This project represents a new collaborative interaction between Prof. Fuchter and Prof. Hammond. Both supervisors have a strong track record for multidisciplinary cancer science and supervising doctoral and undergraduate students. The student will be based jointly in the Fuchter lab (Department of Chemistry) and Hammond lab (Department of Oncology), with strong links to the Peck group. The main focus of this rotation will be to focus on the medicinal chemistry of our current lead molecule (P-3). The student will have the opportunity to learn a wide range of techniques spanning synthetic chemistry and medicinal chemistry and chemical biology. Towards the end of the placement, the student will have some exposure to molecular and cell biology techniques (tissue culture, protein analysis, use of hypoxia chambers, etc.)

Ideal student background: The student will require an undergraduate degree in Chemistry, ideally including theoretical and practical expertise in chemical biology and/or medicinal chemistry. They should have a strong motivation towards multidisciplinary research at the interface between chemistry and biology.

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

Primary Supervisor: Kezia Gaitskell
Additional Supervisors: Gillian Reeves
Eligibility: Track 3 and Track 4 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.

Rotational Project: Developing a genetic prediction model for progression from in-situ to invasive breast cancer

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 tissue samples of 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].

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. Exome sequencing data from blood samples are available for a subset of MWS participants, including for >12,000 women with invasive breast cancer, >2000 women with DCIS alone, and 525 with both.

In this 6-month project, we aim to investigate genetic predictors of progression from in-situ to invasive breast cancer, by comparing genetic differences, based on exome sequencing, between women with DCIS alone vs invasive breast cancer in the MWS cohort. These exome sequencing data will be used to develop a common and rare-variant genetic prediction model for progression from DCIS to invasive breast cancer. The ability to predict progression has a variety of possible future clinical applications, including informing prognosis and planning more or less aggressive treatment.

If continuing for the full DPhil, the genetic variants that are identified as key contributors to this genetic prediction model can then be taken forward for further investigation and validation, using tissue samples from the subset of participants from Oxford.

Training opportunities

This project will involve training in general epidemiological methods, and specifically in the analysis of exome sequencing data.

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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21. 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 3 and Track 4 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.

Rotational Project: Understand the changes in antigen-specific CD8+ T cell responses during the progression from inflammatory disease to HCC

Key for this project will be the identification of antigen-specific CD8+ T cell responses that occur during the transition from inflammatory liver disease to HCC. We expect to identify common antigens and reactive T cell receptors that become present during this transition 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.

Training opportunities: You will use bioinformatic tools to predict peptides from common antigens and mutated proteins present across patients (such as a-Fetoprotein, Glipican-3, NY-ESO-1, MAGE-A, P53, etc). Following the identification of these peptides you will use blood and tumour tissues obtained from patients with inflammatory liver diseases and early HCC to stimulate T cells and perform ELISpot assays and T cell receptor cloning to identify reactive T cells and their cognate T cell receptors.

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

Primary Supervisor: Audrey Gérard
Additional Supervisors: Claudia Monaco
Eligibility: Track 3 applicants are eligible to apply for this project

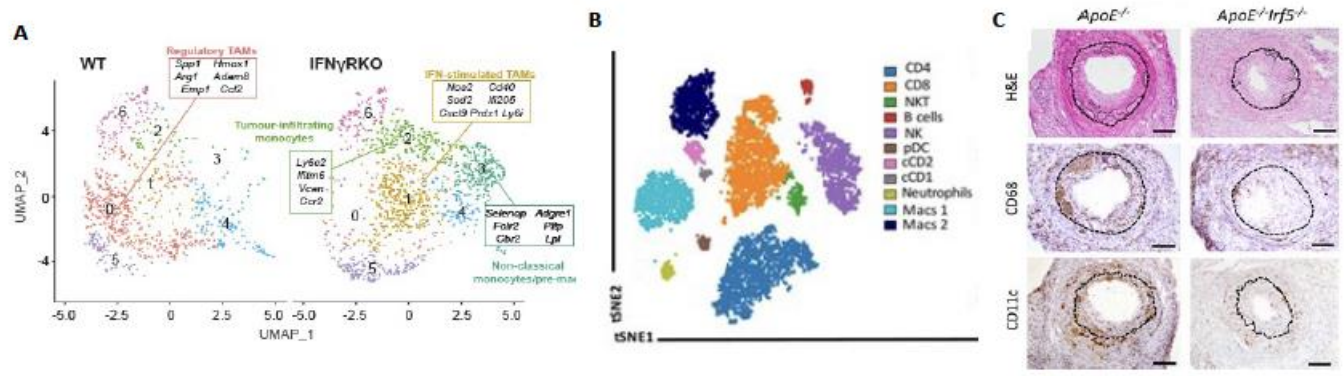
Abstract
Cancer cells can be recognised and killed by our immune system. However, tumours developed strategies to evade the immune system by rewiring immune cell types that are critical for tumour control. In recent years, therapeutics such as immune checkpoint inhibitors emerged to repristinate immune 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 chekpoint 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.

Rotational Project: Relationship between Tumour microenvironment and atherosclerotic cardiovascular disease

Cancer cells can be recognised and killed by our immune system. However, tumours developed strategies to evade the immune system by inhibiting rewiring immune cell types that that are critical for tumour control. In recent years, immune therapeutics emerged to counteract this, called checkpoint blockade. This treatment aims to reinvigorate immune cells called CD8 T-cells and has 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; and the reason for this is not completely understood. To address this question, we need to understand how the tumour microenvironment (TME) influences distal immune responses, either systemic, or at the atherosclerotic sites. This 6-month project aims at characterising the immune types and immune mediators that are present in the bone marrow, blood and atherosclerotic plaques depending on the type of TME elicited.

Atherosclerosis prone mice will be engrafted with different cell lines which are known to induce different TME. Using Olink's technology, you will characterise which cytokines are produced at the different sites, and whether this is influenced by the type of TME elicited. You will then analyse the immune landscape at those sites. To do so, you will learn state-of-the-art immunological procedures and use techniques such as spectral flow cytometry, CYTOF, Elisa or Legendplex.

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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23. 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.

Rotational Project: Uptake of treatment and clinical outcomes following treatment of individuals diagnosed with prostate cancer in the NHS in England

Prostate cancer is one of the most prevalent cancers and causes of cancer death worldwide. The development and aggressiveness of prostate cancer is influenced by factors including ethnicity, family history, age, environmental and social variables. Prostate cancer outcomes are affected by significant health inequities, for example men of Black ethnicity have higher incidence and mortality and are more likely to be diagnosed at late stage at younger age, than those of White ethnicity; these disparities are larger than for any other common cancer. Prostate cancer is commonly treated with surgery and radiotherapy for localised disease, and combination of anti-androgen (hormone) therapy, chemotherapy and radiotherapy for advanced or metastatic disease. Studies have shown sociodemographic variation in access to cancer treatments, such as radiotherapy and chemotherapy, and outcomes in the UK for multiple cancers, including poorer access and outcomes for individuals in deprived areas.

This project will determine the uptake of surgery, radiotherapy, chemotherapy and hormone therapy in a cohort of individuals with diagnosis of prostate cancer in the QResearch primary care database, and explore sociodemographic factors affecting treatment uptake, including age, ethnicity, socioeconomic deprivation and geographic region. The QResearch database contains longitudinal data of over 35 million patients, and is linked to NHS secondary care datasets, including Hospital Episode Statistics, National Cancer Registration and Analysis Service, Systemic Anticancer Therapy (SACT) and Radiotherapy Dataset to enable outcome determination. The project will also characterise the outcomes of patients with prostate cancer, including duration of survival, mortality, and unplanned hospital admissions following treatment, as well as factors influencing these. The outputs of this project will provide valuable insights on health inequalities in prostate cancer care and outcomes, which will inform policy makers and healthcare professionals and guide strategies to mitigate these.

Training opportunities

The student will have training and gain transferable skills in analytical methods, including use of large electronic health record data, cleaning and pre-processing of data and statistical analysis using Stata, and gain experience of the QResearch project lifecycle, in preparation for full project. The student will be able to access dedicated training and resources on Stata. The student will be able to gain experience in development of research protocol and analysis plan, project management and presentation of research. The student will be supported to develop as an independent researcher within the multidisciplinary Primary Care Epidemiology Group

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

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24. Using immune fitness as a predictor of cancer risk and progression. – Chris Holmes

Primary Supervisor: Chris Holmes
Additional Supervisors: Xin Lu
Eligibility: Track 3 and Track 4 applicants are eligible to apply for this project

Abstract
The immune system is intricately linked to the initiation, progression, and treatment of cancer. Cancer incidence increases with age, as the immune system declines, whilst immunosuppressed individuals, such as those having undergone transplants, are at greater risk of developing cancer. Previous infection history is also closely linked to cancer initiation, with 13% of cancers globally being attributed to infectious agents: for example, there are known associations between stomach cancer and infection by both H. pylori and Epstein-Barr Virus (EBV), whilst Hepatitis C and B are associated with a higher risk of liver cancer.
Thus, 'immune fitness' – how effectively the immune system surveys cancer cells and establishes, maintains, and regulates an appropriate response – influences multiple stages in cancer and can be used to inform earlier diagnosis and treatment stratification for improved patient survival and public health benefits. Accurate assessment of immune fitness, and subsequent development of an immune fitness "score", must take into account a multitude of factors, including disease history, lifestyle and genetics. A key source of such information is the UK Biobank, a large-scale biomedical database containing genetic information and biological samples from 500,000 UK participants.

Integrating the genetic and biochemical data obtained through the UK Biobank (UKB) will require machine learning data reduction (feature representation learning). For a subset of UKB where we have GP records, we will combine features with the wealth of information contained in those participants' GP records (disease history, infection history, prescriptions). This will provide enhanced representations of a patient's immune fitness, and to predict the risk of cancer risk and progression. The integration and analysis of these large, multimodal datasets is a key challenge, and will require advanced techniques including Big Data approaches and artificial intelligence. This project will focus on developing such techniques and the pipelines required to generate immune fitness scores and predictive methods for cancer risk and progression. The AI approaches will utilize ideas from multi-task learning to jointly learn features that associated with immune compromise as well as cancer outcomes. The co-learning of features is expected to lead to more robust, generalizable, immune fitness features that are predictive of cancer risk.

Research objectives and proposed outcomes
This project will investigate and characterise the links between immune fitness and cancer initiation, progression, and susceptibility of patients to respond or not to immunotherapeutic agents. Specific areas of study will include:

1. **Cancer risk in the general population, using immune fitness as an indicator;**

Drawing upon the wealth of data available through the UK Biobank cohort, factors such as biological age, immune profiles and serological infection history will be used in conjunction with statistical machine learning methods, including neural transformer architectures for multimodal data, to develop a generalised immune fitness score, allowing to identify individuals at high cancer risk and to target preventative and screening measures accordingly.

2. **Prediction of progression from pre-cancerous conditions to malignancy, using immune fitness as an indicator;**

To enhance existing surveillance strategies for pre-cancerous conditions (which can be operator-dependent, resource-intensive and costly), we aim to develop cancer-specific immune fitness scores to identify those individuals most likely to progress to malignancy. For this aim, we will focus on hepatobiliary and gastrointestinal cancers, as these cancers are the most common infection-caused cancers, are often associated with pre-malignant inflammatory conditions, and have outcomes which are greatly improved by the early detection of progression. In addition to the data within the UK Biobank, we have extensive collaborative networks which will provide access to data from high-risk and early cancer cohorts in hepatocellular cancers (e.g., DeLIVER programme) and gastrointestinal cancers (e.g., in collaboration with the Translational Gastroenterology Unit, Oxford University Hospitals). These cohorts have extensive deep phenotyping data available, including genetic, epigenetic, and multimodal imaging data. A cancer type-specific immune fitness score will integrate the above to inform those patients most likely to progress.

3. The use of immune fitness markers for the stratification of cancer patients for immunotherapy.

Immunotherapy is part of standard care for cancers such as melanoma, lung cancer, and head and neck cancers (HNC). Interestingly, patients with HPV+ HNC or EBV+ gastric cancer tend to respond better than non-infected individuals. However, even in viral-caused cancers, only a subset of patients benefit from immunotherapy and immune toxicity is a significant consideration, suggesting that immune fitness and the tumour microenvironment need to be considered to stratify patients for immunotherapy. In a cancer associated with inflammation rather than viral infection, namely oesophageal cancer, we will supplement immunogenetic, immune cell epigenetic signatures and infection history with existing tumour tissue multi-omic data from a cohort of 75 combination PD-L1 immunotherapy-treated oesophageal cancer patients (LUD2015-001 trial). We will explore the potential ability of features and AI methods from projects 1 and 2 to stratify subjects based on predicted immunotherapy response.

Translational potential

There is a direct translational component to this project, which aims to develop a personalised immune fitness score for patients and integrate this with other predictive methods to assess the risk of cancer initiation and/or progression and to stratify patients for immunotherapy suitability. This has the potential to inform preventative measures according to individual risk and therapeutic options in the case of progression.

Training opportunities

This project is interdisciplinary, involving both biological and statistical components, and will be jointly supervised by Professor Chris Holmes, Department of Statistics and Nuffield Department of Medicine, and Professor Xin Lu, Ludwig Institute for Cancer Research. The student will have opportunities to integrate with the wider scientific and clinical communities in Oxford through established collaborative networks, and with the national and international communities at conferences. The student will benefit from the training and career development programme at the Ludwig Institute, which includes: regular oral, journal clubs, and skills development in writing, data management and public engagement. They will also be part of the Computational Statistics and Machine Learning (OxCSML) group in the Statistics departments, that hosts a seminar series and reading clubs in technical aspects of machine learning.

Rotational Project

The immune system is intricately linked to cancer initiation, progression, and response to therapy. 13% of cancers are caused by infectious agents, and inflammation exerts an important influence on tumour initiation and progression. Thus, '**immune fitness**' – how effectively the immune system surveys cancer cells and establishes, maintains, and regulates an appropriate response – influences multiple stages in cancer and can be used to inform earlier diagnosis and treatment stratification for improved patient survival and public health benefits. Accurate assessment of immune fitness, and subsequent development of an immune fitness "score", must take into account a multitude of factors, including disease history, lifestyle and genetics. A key source of such information is the UK Biobank, a large-scale biomedical database containing genetic information and biological samples from 500,000 UK participants.

Integrating the genetic and biochemical data obtained through the UK Biobank with the wealth of information contained in those participants' GP records (disease history, infection history, prescriptions) will enhance our ability to assess a patient's immune fitness, and to predict the risk of cancer risk and progression. The integration and analysis of these large, multimodal datasets is a key challenge, and will require advanced techniques including Big Data approaches and artificial intelligence. This project will focus on developing such techniques and the pipelines required to generate immune fitness scores and predictive methods for cancer risk and progression.

Training opportunities

This project is multidisciplinary and as such the candidate will receive training in both dry lab and wet lab techniques. A large component of this project will be the integration and analysis of large, multimodal data (e.g., from the UK Biobank), and the candidate will be trained in big data, statistical and artificial intelligence approaches in order to achieve this. The student will have opportunities to integrate with the wider scientific and clinical communities in Oxford through established collaborative networks, and with the national and international communities at conferences. The student will benefit from the training and career development programme at the Ludwig Institute, which includes: regular oral, journal clubs, and skills development in writing, data management and public engagement. They will also benefit from training and collaboration opportunities as part of the OxCSML group in Statistics.

References

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25. Develop a neoantigen-like, ROP-based vaccine targeting SVN (ROP-SVN) to boost host immune responses. – Shisong Jiang

Primary Supervisor: Shisong Jiang
Additional Supervisors: Wenshu Lu
Eligibility: Track 3 and Track 4 applicants are eligible to apply for this project

Abstract
Tumour-associated antigens (TAAs) are prevalent in cancer cells and are potential targets for cancer vaccines. However, TAAs are often viewed as self-antigens by the host, leading to reduced immunogenicity when used in vaccines. Furthermore, wildtype-TAAs (WT-TAAs) are oncoproteins and tumourigenic, rendering them unsuitable for direct use in cancer vaccines. We aim to address these challenges by developing recombinant overlapping peptides (ROP)-based cancer vaccines to bolster host immunity. ROPs rearrange WT-TAA sequences to preserve T and B cell epitopes while abolishing the conformation and functions of oncoproteins, effectively transforming a WT-TAA into a safer and more immunogenic, neoantigen-like vaccine. Our goal is to develop mRNA-based ROP vaccines, offering advantages such as easy manufacturing and fewer hurdles for protein purification and refolding. We will evaluate their efficacy compared to protein-based counterparts. Additionally, we will assess the combination of other immunotherapies with ROP-based vaccines, aiming to optimise the cancer therapeutic strategy.

Research objectives and proposed outcomes
Immunotherapy has emerged as a promising strategy for treating cancer by harnessing the patient's immune system to target tumour cells. This approach offers enhanced specificity and efficacy, either alone or in combination with traditional treatments like chemotherapy and radiation therapy. One facet of immunotherapy involves cancer vaccines, which introduce tumour-specific antigens (TSAs) or tumour-associated antigens (TAAs) to stimulate an immune response against tumour cells bearing these antigens (1).

While TSAs are appealing targets, they are limited in their applicability across various cancer types and are typically associated with tumours caused by viral infections or mutation-related neoantigens. In contrast, TAAs are more widely prevalent and thus promising targets for a range of tumours. However, cancer cells can evade immune detection, as TAAs may be perceived as self-antigens, leading to immune tolerance and reduced response. Additionally, many TAAs are oncoproteins, which might not be suitable for direct development into vaccines due to the potential risk of tumourigenesis (2).

This project aims to address these challenges by focusing on the tumour-associated antigen survivin (SVN), which is over-expressed in most tumour types and plays a crucial role in cell mitosis and apoptosis inhibition (3).
Our strategy is to develop an mRNA-based, neoantigen-like SVN vaccine with the following **objectives**:

1) Develop an mRNA ROP-SVN vaccine to boost host immune responses.
2) Compare the mRNA ROP-SVN with the peptide/protein form ROP-SVN.
3) Explore combination therapy of mRNA-ROP-SVN with other immunotherapies.

Our previous studies showed that 15-30-mer peptides can access both MHC class I and II pathways, stimulating CD8+ and CD4+ T cells, unlike protein-based immunogens (4, 5). Building on this, we designed recombinant overlapping peptides (ROPs) to cover the complete sequence of a target protein, presenting all potential T cell

epitopes (4,5,6,7). ROPs, lacking the conformational structure of wild-type proteins, avoid the toxic functions of some oncoproteins, like survivin.

Neoantigen vaccines show great promise but identifying suitable neoantigens is challenging (8). Recreating a TAA like SVN through ROP design alters the structure while preserving T and B cell epitopes, transforming ROP-SVN into a neoantigen-like entity to enhance immunogencity and yet mitigating its toxicity.

Preliminary results: OVM-200 (protein/peptide form of ROP-SVN), has completed preclinical testing and is in a Phase 1 clinical trial with encouraging results (9).

Expected results:
Production of mRNA-ROP-SVN and Protein-ROP-SVN, with mRNA-ROP-SVN formulated with lipid nanoparticles (LNPs).

1) In vitro and in vivo antigenicity evaluation of the protein and mRNA forms of ROP-SVN, including immunizing mice and measuring antibody and T cell responses.
2) In vivo efficacy assessment of the protein and mRNA forms of ROP-SVN using the B16-SVN-based xenograft mouse model.
3) Combined cancer therapy utilizing mRNA-ROP-SVN with other therapies.

Translational potential of the project.
The project is highly translational and, if successful, will provide an affordable, safe, and effective immunotherapy for cancer patients.

Training opportunities
The student will receive training in the following areas:

1) mRNA Technology: Gain experience in in vitro mRNA synthesis.
2) Vaccine Development: Involve in ROP-based therapeutic vaccines from Jiang Lab, which has developed a product currently in Phase I clinical trials.
3) Protein Sciences: Learn protein expression, purification, and assessment techniques, backed by years of lab expertise.
4) Mouse Experiments: Conduct essential mouse model experiments for vaccine research.
5) Immunoassays: Develop fundamental skills in ELISA and ELISPOT techniques.
6) Lipid Nanoparticle (LNP) Formulation: learn the technology necessary for mRNA vaccines.
7) Antigen Presentation and Evaluation Techniques: Focus on Jiang Lab's specialised research area.

Rotational Project: In Vitro Antigen Presentation of a Neoantigen-Like, ROP-Based mRNA Vaccine Targeting Survivin (mRNA-ROP-SVN)

Tumour-associated antigens (TAAs) are prevalent in cancer cells and present potential targets for cancer vaccines. However, TAAs are often recognised as self-antigens by the host, leading to reduced immunogenicity when used in vaccines. Additionally, wild-type TAAs (WT-TAAs) are frequently oncoproteins and tumourigenic, making them unsuitable for direct use in cancer vaccines.
To address these challenges, we will utilise recombinant overlapping peptides (ROP)-based cancer vaccines to bolster host immunity. ROPs rearrange WT-TAA sequences to preserve T and B cell epitopes while abolishing the conformation and functions of oncoproteins, effectively transforming a WT-TAA into a safer and more immunogenic neoantigen.
For the 6-month rotation project, our goal is to develop mRNA-based ROP vaccines, which offer advantages such as ease of manufacturing and fewer hurdles in protein purification and refolding. We will evaluate the antigen presentation of the vaccine in vitro using mouse and human dendritic cell lines, performing assays of cytokine profiles and antigen presentation

Training opportunities

The student will receive training in the following areas:

1) mRNA Technology: Gain experience in in vitro mRNA synthesis.
2) Antigen Presentation and Evaluation Techniques: Focus on Jiang Lab's specialised research area.
3) cytokine assays

Ideal student background: The student should have a strong knowledge in molecular biology, immunology, and oncology, with relevant practical experience in immunoassays, animal studies, and molecular biology. This diverse skill set will enable the successful delivery of this innovative cancer vaccine development project

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(Shisong Jiang Lab)

26. A statistical analysis of captured p53 antibodies – Christiana Kartsonaki

Primary Supervisor: Christiana Kartsonaki
Additional Supervisors: Jason Davis
Eligibility: Track 3 and Track 4 applicants are eligible to apply for this project

Abstract
The tumour suppressor gene TP53 is mutated in over 50% of human cancers, leading to uncontrolled proliferation of cells. The accumulation of abnormal p53 protein results in the body activating an immune response to produce antibodies that recognise p53 in the blood. These antibodies are attractive biomarkers for early cancer detection since, contrary to the many versions of mutated p53 protein in different cancers, the p53 antibody structure is relatively consistent and so one detection assay can pick up a wide range of cancers. Additionally, p53 antibody levels increase to a much greater extent than the mutated p53 protein and so they are easier to detect over the background level. We have developed an electrochemical sensor platform that utilised specific peptide sequences that mimic a region of the p53 protein that is rarely mutated (Kang et al. 2024). When integrated into the surface of manipulatable nanoparticles these enable the isolation of antibodies from blood serum prior to very sensitive amplified quantification.

This project will optimise the nanoparticle surface chemistry and associated microfluidics/magnetic fields to refine the specificity of this target capture and will apply these analyses to plasma samples of patients with cancer and potentially healthy controls to assess the extent to which p53 can be detected in patients whose tumours are likely to harbour mutations in TP53. It will include training in detailed statistical analyses.

Research objectives and proposed outcomes
Plasma proteomics have shown great promise for use in risk prediction and early detection of cancer in asymptomatic individuals (Kartsonaki et al. 2022; Papier et al. 2024). Of particular interest is p53, the protein associated with the tumour suppressor gene TP53, of which both germline and somatic mutations are implicated in many types of cancer.

The accumulation of abnormal p53 protein results in the body activating an immune response to produce antibodies that recognise p53 in the blood. These antibodies are attractive biomarkers for early cancer detection since, contrary to the many versions of mutated p53 protein in different cancers, the p53 antibody structure is relatively consistent and so one detection assay can pick up a wide range of cancers. Additionally, p53 antibody levels increase to a much greater extent than the mutated p53 protein and so they are easier to detect over the background level. We have developed an electrochemical sensor that uses nanoparticles coated in peptide sequences from a region of the p53 protein that is rarely mutated (Kang et al. 2024). These p53 peptide-coated nanoparticles selectively bind to p53 antibodies from blood serum, which are then isolated and presented to the sensor for quantification.

The next step to assess the potential utility of this assay in detecting cancer earlier or predicting future risk of developing cancer would include validation of the method in stored plasma samples of patients with cancers which are likely to harbour somatic mutations in TP53, as well as patients with germline alternations or TP53 mosaicism. Subsequently studies comparing patients with cancer to healthy control participants may be conducted, with the ultimate aim of assessing the utility of the assay in asymptomatic participants in order to detect p53 antibodies before a tumour becomes apparent, such as samples from large-scale prospective cohort biobank studies.

This project will form the basis of an entirely new collaboration between Prof. Jason Davis (Department of Chemistry), who has developed the p53 assay, and Dr Christiana Kartsonaki (Nuffield Department of Population Health), who has expertise in the analysis of proteomics and other biomarkers in cancer risk prediction and early detection.

Translational potential

The project has the potential to lead to the development of a biomarker for the early detection of a number of cancers, as well as potentially useful in the monitoring of cancer among patients with specific cancer types. It may also be helpful in the design of future precision prevention clinical trials.

Training opportunities

The student will receive training in electrochemical assays, microfluidics, nanoparticle chemistry, statistics, cancer epidemiology, presentational skills and study design.

Rotational Project

The tumour suppressor gene TP53 is mutated in over 50% of human cancers, leading to uncontrolled proliferation of cells. The accumulation of abnormal p53 protein results in the body activating an immune response to produce antibodies that recognise p53 in the blood. These antibodies are attractive biomarkers for early cancer detection since, contrary to the many versions of mutated p53 protein in different cancers, the p53 antibody structure is relatively consistent and so one detection assay can pick up a wide range of cancers. Additionally, p53 antibody levels increase to a much greater extent than the mutated p53 protein and so they are easier to detect over the background level. We have developed an electrochemical sensor platform that utilised specific peptide sequences that mimic a region of the p53 protein that is rarely mutated (Kang et al. 2024). When integrated into the surface of manipulatable nanoparticles these enable the isolation of antibodies from blood serum prior to very sensitive amplified quantification.

This project will optimise the nanoparticle surface chemistry and associated microfluidics/magnetic fields to refine the specificity of this target capture and will apply these analyses to plasma samples of patients with cancer and potentially healthy controls to assess the extent to which p53 can be detected in patients whose tumours are likely to harbour mutations in TP53. It will include training in detailed statistical analyses.

Ideal student background: This project would be suitable for a student with an interest in biochemistry and cancer biology, chemistry, as well as interest in developing interdisciplinary skills including data analysis.

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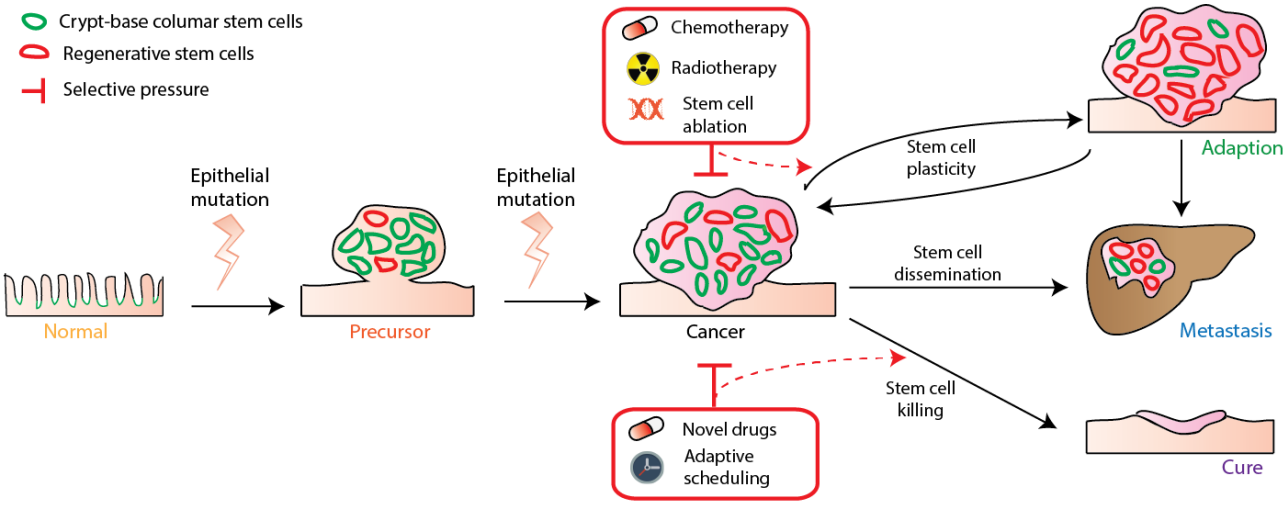
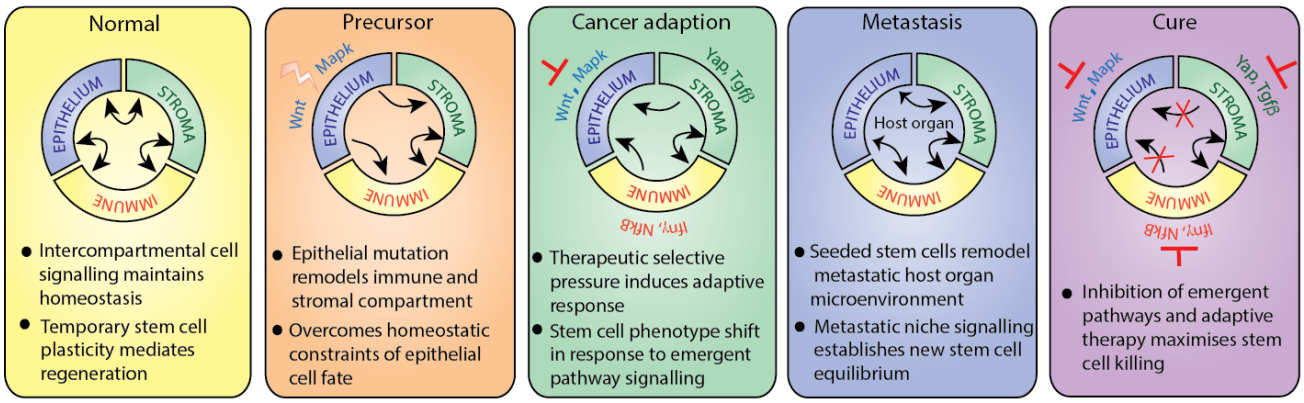
27. Manipulating the stem cell niche to prevent cancer cell adaptive plasticity – Simon Leedham

Primary Supervisor: Simon Leedham
Additional Supervisors: Helen Byrne
Eligibility: Track 3 and Track 4 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.

Rotational Project: Mapping the cancer stem cell niche in mouse and human colorectal cancer

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 and determine the key cellular interactions that regulate different stem cell phenotypes and functionality

Training opportunities

In a rotation project track 3 and 4 candidates will work within a multidisciplinary mathematical and biology team environment to learn the fundamentals of spatial biology image generation, cell segregation, and advanced mathematical analysis and interpretation. Students will be supported to learn and apply our developed in-house software for multiscale spatial analysis and apply this to novel spatial biology datasets.

28. 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 3 and Track 4 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.

Rotational Project

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. Lipid nanoparticles (LPNs) are the main clinically approved carrier for mRNA vaccines formulations; however, they have limited shelf-life and their immune effects are not completely understood, leading to an increased risk of adverse effects. Biodegradable polymers are an effective alternative delivery agent for RNA delivery. They are reducible and easily cleared and possess tunable surface properties for improved endosomal escape. Additionally, lipid-like polymeric constructs (lipidoids) have been developed which incorporate the advantages of both polymeric and lipid delivery systems. Lipidoid formulations which readily transfect skin have not been demonstrated; we hypothesize that polylipidoid nanoparticles can be identified which effectively transfect skin, comparing to standard LNP as a benchmark. This will be tested by synthesizing and screening nanoparticles with different chemistries and characterizing these using *in vitro* assays. We aim to identify suitable polylipidoid nanoformulations for the intradermal delivery of mRNA cancer vaccines through microneedles patches.

Training opportunities

Depending on the student's interest and expertise, they will have the opportunity to receive specialised training in polymer chemistry for the synthesis of a combinatorial library of polylipidoids. They will be guided and supported as necessary by experienced chemists within the Stevens group. The student will focus on the fabrication and characterisation of polylipidoids nanoparticles. 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 learn how to evaluate transfection efficiency both in vitro and in ex-vivo human skin explants.
This 6-months rotational project will provide foundational knowledge and skills for the subsequent DPhil project, aimed at optimising the formulation of polylipidoid nanoparticles for the effective transfection of dermal dendritic cells; optimising their intradermal delivery through microneedles patches and evaluating their efficacy in inducing tumour-specific cytotoxic T-cell response in murine models of cancer.

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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29. The role of hypoxia signalling in tumour microenvironment regulation – Xin Lu

Primary Supervisor: Xin Lu
Additional Supervisors: Chris Schofield
Eligibility: Track 3 and Track 4 applicants are eligible to apply for this project

Abstract
Over 80% of cancers arise from epithelial cells. However, cell fate is not only determined by the cancerous cell but also by the environment in which tumour cells grow, including the surrounding blood vessels, extracellular matrix, immune cells and signalling molecules. This tumour microenvironment (TME) has an important role in cancer progression and responses to therapeutic interventions. Hypoxia – low levels of oxygen – occurs in many tumour environments and can have a major impact on responses to cancer therapy. For example, hypoxic conditions can cause resistance to standard treatments such as radiotherapy. Thus, there are ongoing efforts to manipulate oxygen levels and cellular hypoxia responses to increase therapeutic efficacy. However, we need a better understanding of the crosstalk between hypoxia pathways, other key cancer signalling pathways and cells in the tumour microenvironment – particularly immune cells – to ultimately achieve better outcomes for patients. This project will harness the power of state-of-the-art technologies that provide high spatial and temporal resolution of gene expression and cellular signalling to dissect interactions between cancer cells and their environment. The focus will be on the crosstalk of hypoxia sensitive and response pathways and signalling from the crucial tumour suppressor protein p53, particularly addressing how specific regulatory proteins influence the interplay between immune cells and tumour cells.

Research objectives and proposed outcomes
p53, the tumour suppressor known as the 'guardian of the genome', is inactivated by mutation or other repressive mechanisms in almost all human cancers. As a transcription factor, it regulates myriad cellular pathways and controls cellular life-or-death decisions. Similarly, the HIF factors are transcription factors that control numerous aspects of cellular and physiological behaviour in response to oxygen levels, which are often altered in tumours. Although we know that these two essential regulatory networks intersect, how do they act together in the tumour microenvironment? How does activation of one or both together influence tumour initiation and progression? Are these influences cell autonomous or due to interactions with the immune system?

This project will tackle these cancer relevant questions using the latest strategies to resolve biochemical changes within and outside cells at high spatial and temporal resolution, such as single cell sequencing, metabolomics, proteomics, and highly multiplexed tissue imaging. Prof Lu's group have recently made intriguing discoveries linking regulators of HIF with regulators of p53 and implicating immune cells in tumour suppressive effects via these pathways, providing a strong scientific foundation for this project. Further work, in collaboration with the Ratcliffe group, has investigated the role of Factor-Inhibiting HIF (FIH), a regulator of HIF, in immune homeostasis and its cross-talk with the tumour microenvironment to promote cancer cell growth, using an "Outside-In" in vivo model whereby FIH is deleted from cells of the TME. Also, in a cross-disciplinary collaboration with Hagan Bayley (Department of Chemistry), Xin Lu's group is developing ex vivo 3D multi-cell type co-culture organoid models with realistic tissue architectures. These will be an innovative model for assessing the roles of hypoxia and p53 pathway regulators in cell-cell interactions, mimicking the tumour microenvironment. Prof Schofield is a world-leading expert on the HIF pathway and its enzymatic regulation by and iron and O2 using enzymes, and has expertise on the development and application of small molecule inhibitors targeting regulators of the HIF system.

Translational potential of the project

The longer-term outcomes of this project will support progress in enhanced cancer treatment efficacy, through improved knowledge of key cellular and extracellular interactions that are known to be triggered by standard therapies, for example, the influence of hypoxic conditions and immune cell-tumour interactions on responses to therapy. The use of small molecule inhibitors to manipulate oxygenases involved in the HIF pathway, the regulation of expression, and the extracellular matrix will shed light on potential therapeutic avenues to be pursued, and the results of this project will feed into current clinical efforts to manipulate the hypoxic response to improve patient outcomes to therapy.

Training opportunities

This is a lab-based project and the successful candidate will receive training to become proficient in the use of a wide range of molecular and cellular biology techniques. This includes DNA, RNA and protein analysis methods. Genetic manipulation, in vivo models, flow cytometry and other approaches including, but not limited to, tissue culture, in situ hybridisation, confocal microscopy, time-lapse imaging and FACS. The student will have opportunities to integrate with the wider scientific and clinical communities in Oxford through established collaborative networks, and with the national and international communities at conferences. The successful candidate will also benefit from a wide range of training within the Ludwig Institute, including regular oral presentations, journal club, external seminars, and opportunities to participate in public engagement activities.

Profs Xin Lu and Chris Schofield are internationally-renowned experts in p53 and the hypoxic response, respectively, so the student will be supported by ideal scientific leadership and expertise. Both supervisors have mentored numerous DPhil students, to successful graduation.

Both have established collaborative networks throughout Oxford and beyond, and can enable the student access the infrastructure to make this project a success, including at the Ludwig and the Department of Chemistry. The student will be given training in the necessary cell and molecular biology techniques including single cell genomics, 3D cellular co-culture and multiplex cell-resolution imaging approaches. Chris Schofield is a world leading expert on the HIF pathway and the regulation of the HIF-mediated response, in particular through the use of small molecule inhibitors of enzymatic regulators. The Schofield lab will provide training in proteomics, X-ray crystallography, biological mass spectrometry, molecular biology, kinetics and organic synthesis/medicinal chemistry, as required for the project's evolution. As this is an interdisciplinary project, the successful candidate will benefit from training available to them in both biological and chemical techniques.

The student will also benefit from the training and career development programme at the Ludwig Institute, which includes: regular oral, journal clubs, and skills development in writing, data management and public engagement.

Rotational Project: Investigating hypoxia and p53 pathway crosstalk in the cancer microenvironment

Over 80% of cancers arise from epithelial cells. However, cell fate is not only determined by the cancerous cell but also by the environment in which tumour cells grow, including the surrounding blood vessels, extracellular matrix, immune cells and signalling molecules. This tumour microenvironment (TME) has an important role in cancer progression and responses to therapeutic interventions. Hypoxia – low levels of oxygen – occurs in many tumour environments and can have a major impact on responses to cancer therapy. For example, hypoxic conditions can cause resistance to standard treatments such as radiotherapy. Thus, there are ongoing efforts to manipulate oxygen levels and cellular hypoxia responses to increase therapeutic efficacy. However, we need a better understanding of the crosstalk between hypoxia pathways, other key cancer signalling pathways and cells in the tumour microenvironment – particularly immune cells – to ultimately achieve better outcomes for patients. This project will harness the power of state-of-the-art technologies that provide high spatial and temporal resolution of gene expression and cellular signalling to dissect interactions between cancer cells and their environment. The focus will be on the crosstalk of hypoxia response pathways and signalling from the crucial tumour suppressor p53, particularly addressing how specific regulatory proteins influence the interplay between immune cells and tumour cells. In particular, this 6-month rotation project will focus on the use of organoid cultures as a model system in which to study the crosstalk between p53 and hypoxic pathways and its influence on the TME.

Training opportunities

As a multidisciplinary project, the training opportunities within this rotation project are varied and can be tailored to the successful candidate's research interests. There will be opportunity to learn both biological and chemical techniques. The successful candidate will be trained in all routine biological techniques including cell culture, molecular biology techniques, staining, Western blot, immunofluorescence and microscopy. During this rotation project, the candidate will have the opportunity to become familiar with advanced methods for analysing biochemistry both within and outside of cells, organoid cultures including 3D printing techniques, and to undertake organoid co-culture assays with various cells associated with the tumour microenvironment

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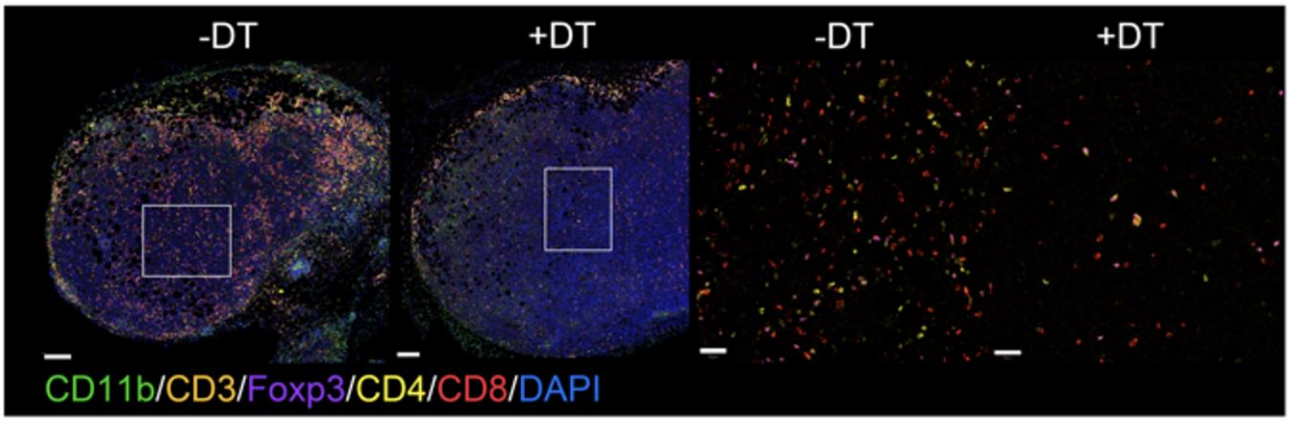
30. Understanding and exploiting cDC1-mediated cross-priming in cancer immunotherapy – Ignacio Melero

Primary Supervisor: Ignacio Melero
Additional Supervisors: Maria Aggleakopoulou
Eligibility: Track 3 and Track 4 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

Rotational Project

Cancer immunotherapy using immune checkpoint inhibitors (ICI) has revolutionised oncology practice, but fails to show clinical benefit in 70-90% of cancer patients. 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. Two subsets of cDC1 cells have been identified depending on expression of the CD103 integrin, which defines the $CD103^+$ migratory cDC1s and a $CD103^-$ resident subtype constitutively located in secondary lymphoid organs. Studies by us and others, using cDC1-deficient mice and XCR1-DTR transgenic mice have demonstrated the necessity of cDC1s in effective cancer immunotherapy. Numbers of cDC1 cells in mice and humans are greatly increased by treatment with a soluble form of Flt3L, that is associated with enhanced efficacy of various immunotherapy approaches. However, the effects of Flt3L, in combination with immunotherapy, on the recruitment, phenotypic activation of cDC1 cell subsets, and their ability to cross-prime $CD8^+$ T cells are unclear. Here, using mouse tumour models and novel sFLT-3-based *in vivo* treatments, we will shed light on these processes. We will elucidate the effects of sFLT-3-based *in vivo* treatments on the recruitment and phenotypic activation of migratory cDC1 cells ($CD103^+$) and of $CD103^-$ resident cDC1 cells, using multi-colour flow cytometry. We will further analyse the ability of migratory $CD103^+$ cDC1 and of $CD103^-$ resident cDC1 cells isolated from tumour draining lymph nodes (DLNs), to cross-present tumour-associated antigens to CD8+ T cells *ex vivo*, after co-culture with Cell-Trace-labelled OT-I OVA- specific $CD8^+$ T cells. The percentages of IFN-γ- expressing $CD8^+$ T cells after stimulation with Brefeldin A, as well as IFN-γ release in culture supernatants will be measured using flow cytometry and ELISA, respectively. The proliferation of Cell Trace-labelled OT-I OVA-specific $CD8^+$ T cells will be measured with flow cytometry. Data from these experiments will be useful and informative for the subsequent mass spectrometry-based immunopeptidome profiling analysis. In parallel, we will use multi-colour flow cytometry or spectral cytometry to assess the expression of presentation, costimulatory, activation and exhaustion markers on tumour infiltrating lymphocytes (focus on naïve and central memory $CD8^+$ T cells) isolated form DLNs and the tumour, from different mouse tumour models. Data from these experiments will be useful for the second research objective of the PhD project, that is the analysis of the immunological synapse (IS) formation between cDC1s and $CD8^+$ T cells.

Training opportunities

The PhD student will be trained to a variety of methods necessary for the facilitation of his future studies. This will involve tissue culture work for the use/maintenance of cancer cell lines, (CT26, MC38, and MC38-OVA cell lines). The student will be given secondary lymphoid tissues (DLNs) and tumour tissue isolated from different tumour models (CT26 and MC38-OVA transfer models) and will learn how to obtain single cell suspensions, followed by cDC1 subset and $CD8^+$ T cell isolation using fluorescence-activated cell sorting or a magnetic labelling system, co-culture of cDC1 subsets with $CD8^+$ T cells and analysis of elicited immune responses using multi-colour flow cytometry.

Ideal student background

You should hold a first 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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31. 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 3 and Track 4 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.

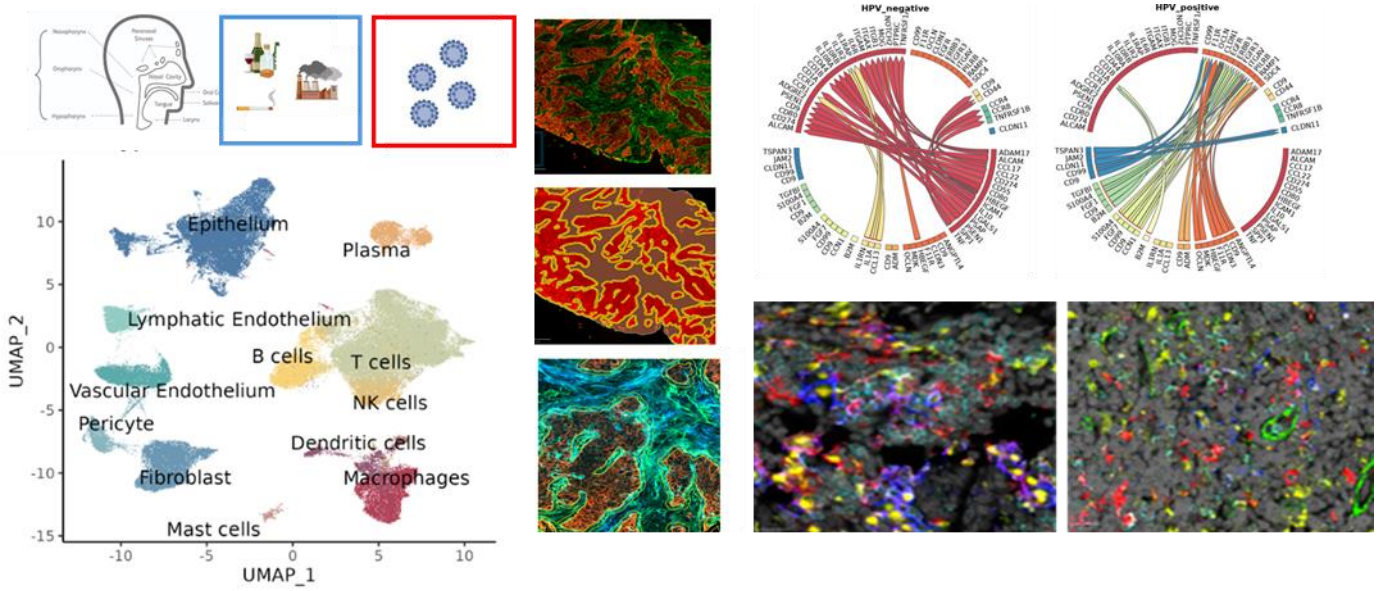

Figure 1. People with head and neck cancer at the same anatomical site, but of different aetiology and treatment response, have distinct matrix patterns that dictate tumor and stromal architecture, and immune infiltration and treatment response.

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.

Rotational Project

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.

Training opportunities

In the 6-month rotation project we will measure mechanical forces across unaffected, tumor adjacent and tumor tissue from immune-infiltrated or -excluded head and neck cancer, and develop imaging panels to assess the expression and localization of mechano- sensitive and responsive genes already identified from existing single cell spatial transcriptomics data sets.

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.

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

Primary Supervisor: Brian Nicholson
Additional Supervisors: Eva Morris
Eligibility: Track 3 and Track 4 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.

Rotational Project: Evidence to inform MCED evaluation.

Multi-cancer early detection (MCED) testing might improve cancer screening and assist doctors in identifying the most appropriate symptomatic patients for cancer investigation. As evidence accumulates about novel MCED technologies regular appraisal of the evidence base is required to inform future studies. The candidate will synthesis published evidence on a chosen MCED or MCEDs to inform the design of a protocol for a retrospective or prospective clinical study or trial to evaluate MCEDs in the community.

Training opportunities

The candidate will develop an understanding of diagnostic research, particularly using data from prospective evaluation of MCED technologies, and develop expertise in evidence synthesis.

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.

33. 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 3 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.

Rotational Project: Nutritional effect on oesophageal cancer phenotype in human tissue avatars

The hippo pathway transduces mechanical forces from the microenvironment to guide proliferation, stem cell behaviour and differentiation. We want to initially verify our more recent work, identifying a signalling pathway recruited to the nuclear envelope in response to acute mechanical force by physically stretching cells or induce hypotonic stress on the cytoskeleton. Our approach is to first determine whether this pathway also responds to differential levels of physiological mechanical stress from increasing extra cellular matrix (ECM) stiffness, by replicating existing finding on increasing levels of crosslinked collagen. We will utilise a combination of human embryonic stem cells, ductal epithelial cells, and pancreatic cancer cells. In line with this we wish to address the extent to which pathological ECM mutations in collagen may impact on the ability to communicate ECM signals to the nuclear envelope. This will allow us to understand the impact of a changing physical microenvironment on signalling to the nucleus. As we now understand that the formation of a nucleoskeleton is important we will also monitor the formation of nuclear actin filaments. We will also determine how the recruitment of SUV39H1/2 and associated repressive histone marks (e.g. H3K9me3) to the nuclear envelope by immunofluorescence and how this is influenced by variations in ECM stiffness. We will aim to also explore the interesting novel discoveries that nuclear f-actin also influences phase separation and potential chromosome boundaries if time allows.

Together this will provide sufficient validation to move the project forward to i) performed genome wide assessment of mobility to determine how specificity is determined, and ii) asses relevance to progression in mouse models of Rassf1^{A133S}, early pancreatic neoplasia (pdx1Kras^{G12D}) and pancreatic ductal organoids. Depending on progress the project will look to examine 'proof of principle' that ECM mechanics influence early progression. Additionally, these aspects provide alternative strands should direction need refinement before initiation of the project due to research progress in the lab.

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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34. 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 3 applicants are eligible to apply for this project

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.

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 use 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).

Rotational Project: Nutritional effect on oesophageal cancer phenotype in human tissue avatars

Patient responses to oesophageal cancer are highly variable, and existing personalisation is limited. We have identified a tumour monocyte signature as predictive of response to immunotherapy, and have tracked the fate of monocytes into further immune cell types using transcriptional single cell RNA-seq data within a clinical trial.

This short project aims to assess develop understanding of monocyte fate in this oesophageal cancer context. Spatial definition of where monocyte populations are in oesophageal cancer, and where their effecting subtypes exert clinical benefit remains unanswered. Using fixed tissue specimens obtained from trial patients we aim to understand the immunological consequences of therapy on the tumour myeloid cell compartment in patients treated with immune activating and standard therapies.

Towards clinical application, we will also aim to better scale monocyte signatures in patients, and how this can be best defined for direct clinical testing using an existing panel of targets.

These results will be highly supportive of *ex vivo* live tissue validation in the avatar culture system as part of an extended DPhil project.

Training opportunities

Students will become familiar with human specimen handling and immunohistochemical techniques, including use of multiplexed systems and image analysis.

35. Generating integrative causal proteomic networks of prostate cancer aetiology - Mahboubeh Parsaeian

Primary Supervisor: Mahboubeh Parsaeian
Additional Supervisors: Tim Elliott
Eligibility: Track 4 applicants are eligible to apply for this project

Abstract
The availability of large-scale proteomics data has the potential to significantly advance our understanding of the molecular mechanisms underlying cancer risk. Many of these datasets are large and complimentary but exist across multiple cohort studies. The integration of these cohort studies will provide a rich resource for understanding the complex interplay of proteins within biological systems in relation to cancer outcomes. In this project, we aim to investigate the effect of the proteome on prostate cancer risk. Proteomics data has been measured across different studies, including the European Prospective Investigation into Cancer and Nutrition (EPIC) and the UK Biobank (UKBB). In the EPIC study, inflammatory proteins (n=368) were measured using Proximity Extension Assay (PEA) technology in 1,434 matched pairs of cases and controls. Additionally, 6,412 proteins were measured using the SomaScan platform in a case-cohort study of 982 cases and 1,494 sub-cohort. Within EPIC-Oxford, we have measured 5,343 proteins using the Olink Explore HT platform in a smaller sample of 623 matched case-control pairs. In the UKBB study, 2,918 proteins were measured using the Olink Explore 3,072 platform in a cohort of 21,481 men, including 1,147 prostate cancer cases.

Preliminary analyses of each dataset will reveal important yet incomplete insights into the role of the proteome in prostate cancer. For example, individual datasets suggest that the immune system plays a critical role at the earliest stages of prostate cancer when intervention may be the most effective. However, a complete realisation of these insights is currently fragmented across the EPIC Olink, EPIC SomaLogic, and UK Biobank platforms. What remains to be achieved is the integration of these datasets in a quantitative manner to provide a more holistic view of the key proteins and biological pathways involved in prostate carcinogenesis and hypothesise potential interventions for precision prevention.

In this project, we aim to develop a comprehensive framework for integrating diverse proteomics datasets from large-scale prospective studies to deepen our understanding of the role of proteins in prostate cancer, but which will be applicable to future studies of molecular traits across cancer risk more generally. Due to differences in study design, the number of proteins, proteomic technologies, sample size, statistical power, and availability of subgroup analyses (such as clinically important cancer sub-types), we will analyse the data separately. A Bayesian network analysis will then be used to identify protein-cancer relationships and estimate their effects within each dataset.[1] We will then integrate these individual network structures into a meta-network, accounting for heterogeneity and uncertainty between datasets. Techniques like network fusion[2] and Bayesian network meta-analysis[3] will be used to harmonise the datasets, and find common information across the different platforms, at the network and pathway level rather than the feature level. Therefore, the results shift our insight from a fragmented understanding on the role of individual proteins to a quantitative estimation of the proteomic networks and pathways of cancer risk that can inform future interventions for prostate cancer.

Besides integrating proteomics data to increase statistical power, we aim to enhance our discovery results by incorporating existing biological knowledge and genetic evidence, to reduce false discoveries and confounding factors. The complexity of proteomics discovery studies arises from complex mechanisms and interactions between proteins within biological pathways, as well as variability within assays[4], especially for proteins at low

abundance in healthy populations but which may be of particular interest for cancer aetiology. Furthermore, the limitations of current statistical models in accurately capturing these underlying pathways, combined with the high dimensionality of proteomics data, exacerbate this issue. Therefore, integrating biological knowledge, such as protein-protein interaction networks and genetic evidence like Mendelian randomisation (MR) and whole genome sequencing, can reduce biases and infer causal networks for proteins and cancer associations. This approach improves the reliability and interpretability of proteomics findings in discovery studies.

Research objectives:

1. Develop robust methods for harmonising and integrating proteomics data generated from different prospective studies to estimate a robust proteomic network and take our understanding of the role of proteome in prostate cancer risk from single proteins to higher resolution biological pathways.
2. Integrate existing biological knowledge and genetic evidence to enhance the accuracy and interpretability of the role of the proteome in prostate cancer risk and facilitate the identification of candidate causal proteins for future translation research in precision prevention.

Proposed Outcomes

1. This project will advance data integration techniques, applicable not only for proteomics and prostate cancer, but also to other types of OMICS data and cancer sites. These methods will address current limitations in statistical models, improve handling complex, highly correlated data, and consider heterogeneity and uncertainty among key biological markers of cancer aetiology.

2. The project's results will advance our current research on the role of inflammatory proteins in prostate cancer. This approach will enable us to map out complex protein interactions and pinpoint inflammatory proteins within these networks as potential discovery targets. The use of genetic evidence will facilitate our ongoing aim to identify causal candidates and establish a credible set of the most important proteins that influence the reduction in prostate cancer risk.

3. The project will foster diverse collaborations among researchers from the Cancer Epidemiology Unit and prominent scientists from data scientists to clinical experts. Tim Elliott, with extensive experience in immunology and cancer research, will help link our discoveries from prospective cohorts to biological insights, providing a clear line of sight from molecular mechanisms to clinical outcomes.

Translational potential of the project

Recent advances in our ability to measure large-scale proteomics has the potential to lead to a paradigm shift in our understanding of cancer aetiology. Our preliminary findings for prostate cancer have already highlighted that there is a key moment in the aetiology of a prostate tumour when heightened immune-surveillance may be of benefit for prevention. However, at present many of our proteomic and proteogenomic resources are siloed across distinct datasets and therefore any single resource provides insight on only a fraction of the underlying biology. We propose that with new methods that are developed as part of this project, we will be able to integrate results from up to 9,000 proteins from two large prospective cohorts with both the wealth of biological knowledge that exists in resources, such as reactome, and with our latest whole genome sequence analyses of protein-protein dependencies, to build causal proteomic networks of prostate tumorigenesis. These networks will enhance our understanding of cancer aetiology for target identification and facilitate the stratification of individuals at high risk for (pre)cancer into precision prevention trials and early diagnosis.

Training opportunities

As part of this project, the student will receive comprehensive training in data integration, statistical modelling, proteomics analysis, and biomarker discovery, using advanced computational tools. They will gain

skills in enrichment analysis, incorporating biological knowledge to validate features, and learn to integrate modular relations in protein networks to enhance findings' interpretability. The program also emphasises scientific communication through conference presentations and paper writing, supported by scientific workshops and networking opportunities.

Rotational Project: Integrating longitudinal proteomic data with immunophenotype at diagnosis

Preliminary work indicates the appearance of proteomic immune- signatures in the serum of individuals that modify cancer risk long before prostate cancer diagnosis. We wish to understand the mechanistic significance of these signatures by investigating the immune landscape of tumour samples taken at diagnosis. The project will therefore generate new spatial and single-cell immunophenotyping data from banked tumour samples, or

use data deposited in the COMBATcancer data (Combined Molecular Analysis of Blood and Tissue in cancer) to investigate correlations between plasma signature and immune reactivity *in situ*.

Training opportunities
This project offers a comprehensive training program designed to equip non-clinicians, focusing on advanced techniques in cancer immunology and proteomics. The student will have full access to experimental approaches to multiplex immunophenotyping, such as spectral flow cytometry, single-cell RNAseq, CITE-seq, and spatial biology. They will also gain hands-on experience with laboratory techniques, bioinformatics tools, and statistical analysis needed for working with spatial and single-cell immunophenotyping for data preparation and analysis.

Ideal student background: The selected individual for this project should have strong quantitative skills, including familiarity with machine learning methods, for data reduction and feature selection, as well as experience in developing models using Bayesian inference. Eligible candidates should have a background in bioinformatics, biostatistics, computational biology, or related disciplines.

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36. Understanding the role of CD4 T follicular helper cells in the formation and maintenance of tertiary lymphoid structures in colorectal cancer - Isabela Pedroza-Pacheco

Primary Supervisor: Isabela Pedroza-Pacheco
Additional Supervisors: Tao Dong
Eligibility: Track 3 applicants are eligible to apply for this project

Abstract
Recent evidence indicates the presence of ectopic lymphoid aggregates or hubs, often termed tertiary lymphoid structures (TLS) resembling germinal centres (GCs) within tumour tissue, is associated with longer survival and better treatment response. However, the mechanisms contributing to TLS formation and maintainance remain incompletely understood. Given the critical role that CD4 T follicular helper (Tfh) cells have in the formation of GCs, we hypothesise that their magnitude, function, and specificity contribute to the initial events of TLS formation and that their modulation may lead to enhanced TLS-mediated anti-tumour responses. This collaborative project between the laboratories led by Dr Pedroza-Pacheco and Prof Tao Dong at CIO and CAMS-COI aims to elucidate key CD4 T cell subsets that contribute to the formation and maintainance of TLS in patients diagnosed with CRC. This research will not only inform our fundamental understanding of the inner works that drive TLS responses but also has clinical potential to leverage CD4 Tfh cells to be used as biomarkers of predictive response and/or develop therapies to enhance or recreate TLS in CRC, thereby improve prognosis.

Keywords: germinal centres, tertiary lymphoid structures, T follicular helper cells.

Research Objectives
Aim 1: Phenotypic and functional characterisation and specificity of CD4 Tfh cells in the tumour microenvironment of CRC patients.
In-depth phenotypic/functional quantification of the immune system in cancer is critical to understanding how Tfh cells interact and potentially shape TLS. To this end, the LFI lab has developed a high-dimensional spectral flow cytometry platform sampling up to 10^7 immune cells from tumours, adjacent tissue, and blood from treatment-naive CRC patients, enabling an in-depth characterisation of Tfh cells at the protein and functional level. Combined with single-cell RNA sequencing and TCR sequencing, we will dissect transcriptional and clonal features of Tfh cells and how they interact with the TME. Using whole-genome sequencing to inform optimisation of antigen-specific assays, we aim to characterise whether Tfh cells are tumour-specific or bystanders driven by previous exposure to viral/bacterial infections by the establishment of CD4 Tfh cell lines using well-established pipelines at the Dong Lab.

Aim 2: Dissecting the functional importance of tumour-specific Tfh cells in TLS.
Using combined spatial transcriptomics, proteomics, and TCR sequencing, we will be able to discern which Tfh subsets discovered in Aim 1 contribute to TLS. Aim 2 will be supported by Prof Marco Fritzsche, director of the Oxford ZEISS Centre of Excellence and expert in network-based approaches. With our combined expertise in physics-based models and network-based algorithms, we will determine spatial Tfh patterns supporting TLS initiation, and formation.

Aim 3: To mechanistically dissect how Tfh cells impact tumour growth during treatment.

With a 20% relapse rate, there is an urgent need to determine the immune response that controls tumour growth. To this end, we will optimise 2D/3D systems to provide spatiotemporal context of Tfh cell contributions in tumour growth by blocking receptor-ligand interactions critical for Tfh cell interactions with B cells, CD8 T cells, and tumour cells.

Translation Potential
Understanding how CD4 Tfh contribute to anti-tumour responses provides an exciting opportunity for their translation into precision immunotherapies and cancer vaccines.

Training Opportunities
High-dimensional flow cytometry assays, T cell assays, 2D/3D functional assays and organoid systems, bioinformatics techniques.

Rotational Project: Development of 2D/3D organoid systems to study how T follicular helper cells contribute to tumour control in colorectal cancer.

Recent evidence indicates the presence of ectopic lymphoid aggregates or hubs, often termed tertiary lymphoid structures (TLS) resembling germinal centres (GCs) within tumour tissue, is associated with longer survival and better treatment response. However, the mechanisms contributing to TLS formation and maintainance remain incompletely understood. Given the critical role that CD4 T follicular helper cells (Tfh) have in the formation of GCs, we hypothesise that their magnitude, function, and specificity contribute to the initial events of TLS formation and that their modulation may lead to enhanced anti-tumour responses. This collaborative project between the laboratories led by Dr Pedroza-Pacheco and Prof Tao Dong at the CIO and CAMS-COI aims to elucidate key CD4 T cell subsets that contribute to the formation and maintainance of TLS in colorectal cancer (CRC). This rotational project will aim to optimise 2D/3D systems to study the role CD4 Tfh in the control of tumour cells, which will form the basis of a 3-year research programme.

This research programme will not only inform our fundamental understanding of the inner works that drive TLS responses but also has clinical potential to leverage Tfh to be used as biomarkers of predictive response and/or develop therapies to enhance/recreate TLS in CRC and improve prognosis.

Training opportunities
High-dimensional flow cytometry assays, 2D/3D functional assays and organoid systems.

Ideal student background: Students with a strong interest and background in cellular immunology, with a focus on CD4 T cell biology. Familiarity with tissue culture, flow cytometry and in vitro cultures is required. Experience in R/Python will be an advantage but not essential.

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Bradley, T., Peppa, D., Pedroza-Pacheco, I., …Borrow, P. & Haynes, B. F. RAB11FIP5 Expression and Altered Natural Killer Cell Function Are Associated with Induction of HIV Broadly Neutralizing Antibody Responses. *Cell* 175, 387-399.e17 (2018).

37. 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 3 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

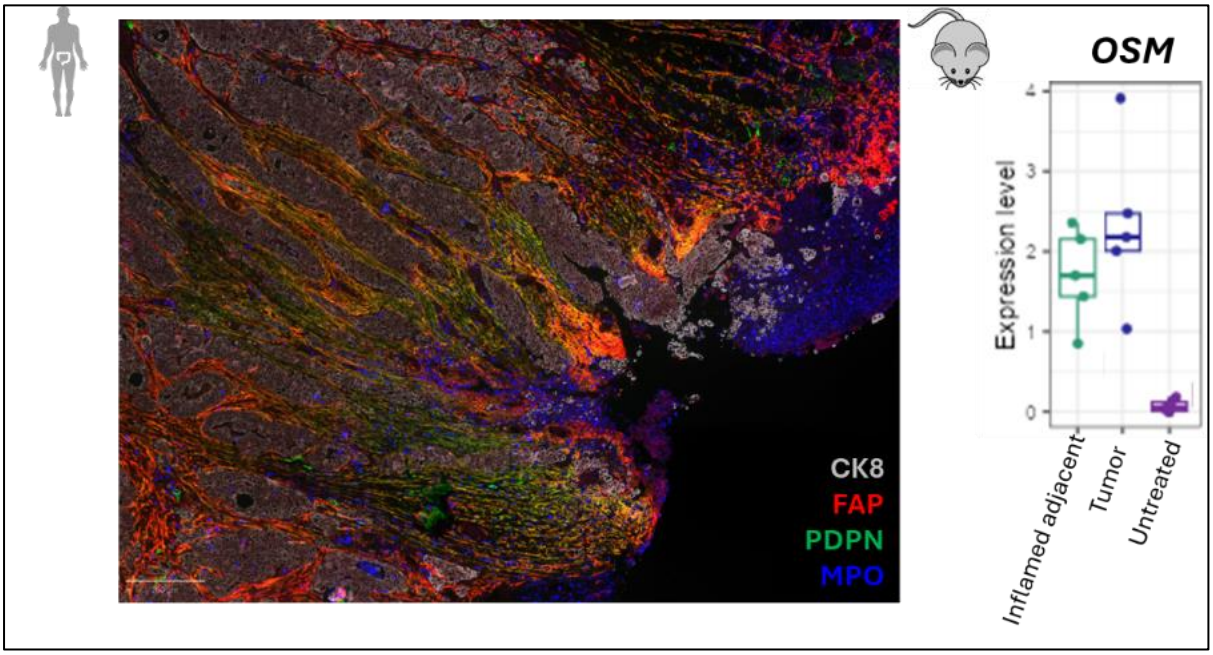

Figure 1. A. Immunohistostaining of resected human CRC tissue showing the presence of neutrophils (Myeloperoxydase MPO) and inflammatory fibroblasts (Podoplanin PDPN, Fibroblast activated Protein FAP) in the tumour (Cytokeratin CK8). B. Transcriptomic expression of *OSM* in the inflamed tissue, tumour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

inflamed adjacent tissue of a colitis associated cancer mouse model (Hh+ Azoxymethane AOm) (Figure 1B). We hypothesise 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.

Rotational Project: Characterisation of Oncostatin M signalling on intestinal stromal cells

Inflammatory Bowel Diseases (IBD) is a chronic inflammatory disease of the gastrointestinal tract characterised by a breakdown of the epithelial barrier leading to ulceration and tissue injury. 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]. OSM signals through OSMR-gp130 and LIFR-gp130 receptors complex, which are differentially expressed by multiple stromal cells including fibroblasts, pericytes, smooth muscle cells and endothelial cells. Signalling activities downstream of OSM include Signal transducer and activator of transcription (STAT)1 and STAT3 activation but the functional relevance of these signalling pathways in the context of epithelial repair is unknown.

Training opportunities
In the 6-month rotation project we will use mouse stromal primary cells (fibroblasts, endothelial cells etc) from OSMR, STAT1 and STAT3 deficient mice to investigate the function of OSM in these relevant cell types. The trainee will learn how to isolate and culture mouse primary stromal cells and will develop a model of epithelial – stromal cells organoids to investigate the effect of OSM on epithelial cell repair. The trainee will also characterise the signalling pathways and the gene program induced upon wound healing in a mouse model of intestinal punch biopsy using multiplex immunofluorescence and transcriptomic.

Ideal student background: We are looking for a highly motivated and intellectually curious candidate with an interest in the intestinal tissue biology, epithelial regeneration, immuno-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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38. Generating real world evidence for patients diagnosed with early onset colorectal cancer - Daniel Prieto Alhambra

Primary Supervisor: Daniel Prieto Alhambra
Additional Supervisors: Eva Morris, Rafael Pinedo-Villanueva
Eligibility: Track 3 and Track 4 applicants are eligible to apply for this project

Abstract
Colorectal cancer has shown an alarming increase in individuals under 50 years of age and is largely unexplained. Evidence suggests there are multiple causes, but it remains unclear what these risk factors are and to what extent these factors explain recent increases in early-onset colorectal cancer and the impact this has and could have in the future on healthcare systems if cases continue to rise.

Bowel cancer screening programmes have been successful with the earlier diagnosis of colorectal cancers for those older than 60 years of age. However, with no screening for those under 50 and a lack of awareness of clinical symptoms this has led to younger patients presenting with more advanced disease and worse survival rates. Furthermore, the increasing cancer rates in younger patients has a knock-on effect on society and healthcare which could be preventable. Therefore, there is a clear evidence gap for research investigating early onset colorectal cancer given the uncertainty around its cause and missed opportunities for prevention and earlier diagnosis necessitating the need to understand why and how these can be reduced to improve care and outcomes.

This project will use real world evidence to quantify and characterise the disease burden of colorectal cancer in the under 50's in the UK, as well as using this data to generate evidence to understand differences in diagnosis and care to see if this is changing over time to highlight any inequities that could be targeted for improvement and how this could be utilised to reduce costs to healthcare services.

Research objectives and proposed outcomes
The aim of this project is to generate real world evidence for early onset colorectal cancer. This will be achieved with the following objectives:

1) Determine and characterize time trends of disease burden and survival in those diagnosed with early onset colorectal cancer using a variety of real-world datasets.
2) To determine the differences in presentation of symptoms, where diagnosis occurred, and treatments of those diagnosed with early onset colorectal cancer compared to older populations and if this impacts survival related outcomes.
3) Use the evidence gathered to determine the impact of increasing cancer cases, delayed diagnosis, more treatments on economic costs to healthcare providers and others and what an improvement in diagnosis and care could lead to for those diagnosed with early onset colorectal cancer.

Translational potential of the project.
The translation potential of the project is substantial as there is good evidence that early onset colorectal cancer is rising, and this could lead to variation in cancer diagnosis and therefore care across the UK. The findings could inform clinical guidelines, public health policies, resource allocation, and patient education efforts, ultimately leading to earlier detection, improved care, and reduced economic burden associated with early-onset colorectal cancer.

Training opportunities
The student will work within the Health Data Science Division gaining research experience and training in epidemiology, health economics, and health data science. The student will be embedded within the international European Health Data & Evidence Network (EHDEN) and Observational Health Data Sciences and Informatics (OHDSI) networks to ensure additional analytical guidance, training and support. There will be opportunities to communicate their findings at national and international conferences and with the public to enrich their studies.

The student will be supported by an experienced and collaborative supervisory team and receive professional mentorship through regular supervisory meetings. The student will be encouraged to attend the variety of seminars and workshops offered by the medical science division to acquire key research and transferable skills to support their research.

Core training consisting of lectures will be provided to give a solid foundation in a broad range of subjects including statistics, epidemiology, health economics and big data analysis. The student will be required to attend a 2-day Statistical and Experimental Design course and the Real-World Epidemiology: Oxford Summer School. Other training in information governance and research ethics will also be provided to ensure appropriate understanding and correct use of patient level health data.

Rotational Project: Descriptive epidemiology of early onset colorectal cancer

Colorectal cancer has shown an alarming increase in individuals under 50 years of age and is largely unexplained. Evidence suggests there are multiple causes, but it remains unclear what these risk factors are and to what extent these factors explain recent increases in early-onset colorectal cancer and the impact this has and could have in the future on healthcare systems if cases continue to rise.

Real-world data, such as medical records, can provide valuable insights into early-onset colorectal cancer. By utilizing this data, we can generate evidence that could improve early diagnosis and increase the number of people surviving the disease in a cost-effective way.

This project will use a variety of real-world datasets across UK and Europe, which the supervisors have access too, to perform a variety of descriptive analysis of early onset colorectal cancer patients. Analyses will include estimating the incidence, prevalence and survival of these patients as well as the large-scale characterisation of comorbidities and medication use before, on and after their diagnosis and if this has changed over time.
These findings will be crucial for healthcare providers and policymakers to gain a better understanding of early-onset colorectal cancer and could inform clinical guidelines, public health policies, resource allocation, and patient education efforts, ultimately leading to earlier diagnoses, improved survival rates, and better quality of life for patients.

Training opportunities
The student will work within the Health Data Science Division gaining hands on research experience and skills training in epidemiology and health data science from the Pharmaco- and Device epidemiology research group.

The student will be embedded within the international European Health Data & Evidence Network (EHDEN) and Observational Health Data Sciences and Informatics (OHDSI) networks to ensure additional analytical guidance, training and support. The student will attend internal training sessions on software packages developed by the team to carry out descriptive studies focussing on estimating the incidence, prevalence, survival and characterisation of young onset colorectal cancer patients (https://darwin-eu.github.io/IncidencePrevalence/)/. Other training in information governance and research ethics will also be provided to ensure appropriate understanding and correct use of patient level health data vital for this project.

The student will be supported by an experienced and collaborative supervisory team and receive professional mentorship through regular supervisory meetings. The student will be encouraged to present at biweekly team meetings as well as other seminars and workshops offered by the medical science division to acquire key research and transferable skills to support their research.

Ideal student background: This project would suit a student with a background in epidemiology, biostatistics, mathematics or engineering at either an undergraduate or master's level. The student will require a good level of programming experience to complete this project. The student should possess understanding of basic statistics and experience in handling/analysing real world data sources such as primary care/secondary care or cancer registry data would be an advantage but not essential as training will be provided. An interest in health data sciences and particularly in oncology is also desirable

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39. 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 3 and Track 4 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.

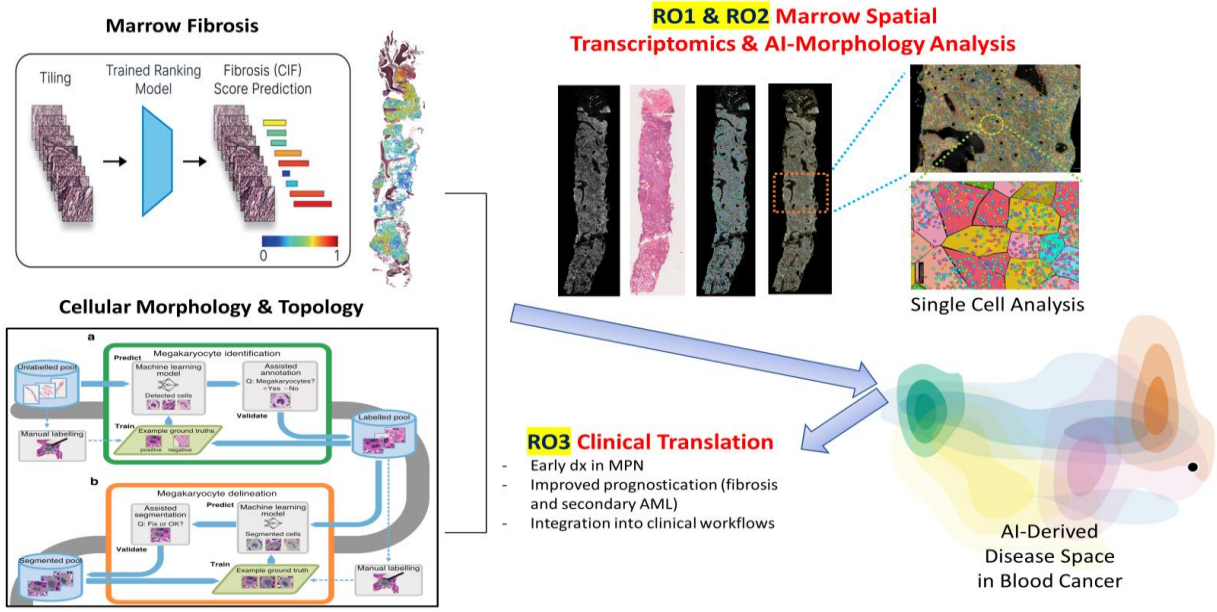

Figure 1. Overview of project workflow

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.

Rotational Project: Integrating bone marrow tissue histology with spatial transcriptomic data

Conventional microscopic assessment of bone marrow biopsies by pathologists involves subjective and qualitative descriptions of important tissue features. These approaches fail to identify important cell-cell and cell-stromal interactions implicated in the initiation and progression of blood cancers in the bone marrow. In response, our group has previously built AI-powered approaches to improve the detection of important morphological features including marrow fibrosis and megakaryocyte cytomorphology & topology. We have also developed and refined in-situ spatial transcriptomic (ST) approaches that enable high resolution detection and characterisation (phenotypic and genotypic) of individual stromal, immune and haematopoietic cell populations in the marrow of intact tissue biopsies taken from patients diagnosed or investigated for blood cancer. We now look to establish computational / bioinformatic approaches to analyse and integrate this ST data with key morphological characteristics of the bone marrow in health and disease. These approaches will then be used to build AI-powered models of bone marrow tissue that can be applied to routine tissue biopsy samples. Outputs from this work will be central to our ultimate aims of identifying robust markers of early-stage disease progression in blood cancer patients that can be translated into standard clinical practice.

Training Opportunities
In addition to the generic training opportunities offered by the Oxford Cancer Centre, rotational students will be provided training in a wide range of tissue diagnostic and analytical techniques that are widely applicable to subsequent DPhil projects. Techniques utilized in our group include conventional microscopy by expert pathologists, immunohistochemistry (IHC) / immunofluorescence (IF) microscopy, and spatial transcriptomics analysis of tissue biopsies. Students will have the opportunity to prepare, run and analyses samples using the *10x Xenium* platform by experts in tissue biopsy processing and interpretation. They will 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 Institute of Biomedical Engineering (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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Quantitative analysis of bone marrow fibrosis highlights heterogeneity in myelofibrosis & augments histological assessment; insights from a phase II clinical study of zinpentraxin alfa. Accepted *HemaSphere, May 2024*.

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Continuous indexing of fibrosis (CIF): improving the assessment and classification of MPN patients. *Leukaemia. 2022.*

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40. Molecular basis for T cell recognition of public neoantigens – Malcom Sim

Primary Supervisor: Malcom Sim
Additional Supervisors: Tim Elliott
Eligibility: Track 3 and Track 4 applicants are eligible to apply for this project

Abstract
T cells can eliminate tumours through detection of neoantigens presented on class I and class II human leukocyte antigens (HLA-I and HLA-II) via the T cell receptor (TCR). Neoantigens derive from protein substitutions resulting from DNA base changes across the genome. Most neoantigens are 'private', derived from the unique complement of mutations found in an induvial tumour. However, a subset of neoantigens are shared between cancers and patients across indications, due to common mutations in key oncogenic pathways. These 'public' neoantigens are derived from some of the most mutated genes in cancer including KRAS, p53 and EGFR. Critically, these neoantigens can be immunogenic and immunotherapies targeting these neoantigens have shown promising effects in early phase trials (Leidner et al., 2022; Tran et al., 2016). Along with partners, the Oxford Centre for Immuno-Oncology is developing an experimental cancer vaccine that targets 16 public neoantigens for use in patients at high risk of recurrence (https://www.ox.ac.uk/news/2024-03-22-new-funding-development-worlds-first-lung-cancer-vaccine). Some public neoantigen TCR complexes have been studied with molecular and structural detail (Sim et al., 2024; Sim et al., 2020; Sim and Sun, 2022; Wu et al., 2020), however we have lack data for many of the antigens targeted by this vaccine. It is critical that we understand as much as possible about how the immune system detects these 'public' neoantigens, to facilitate the development of this vaccine and other immunotherapies. As part of this effort, we will investigate the molecular basis for how 'public' neoantigens are presented by HLA-I and HLA-II molecules and detected by T cells.

Research Objectives
There are two main objectives:

Objective 1: Determine 3D structures of public neoantigen TCR:HLA complexes

There is considerable interest in developing computational methods to predict TCR specificity (McMaster et al., 2024; Sim, 2024).However, the small size and biased nature of the existing structure dataset remains a barrier to accurate computational prediction. Neoantigens often differ from 'self' sequences by single amino acids and therefore present an extreme challenge for TCR discrimination. By determining numerous structures of neoantigen specific TCR-pHLA complexes, we will provide fundamental insights into how T cells detect cancer and discriminate between antigens of highly similar sequences. Futher, these data will provide additional data to community efforts in improving compuntational methods for predicting TCR speificity.

Objective 2: Determine the biophysical properties of public neoantigen TCR:HLA interactions

Previous studies have indicated that bio-physical properties of TCR:pMHC interactions are relevant to understand the immunotherapeutic efficacy (Sim et al., 2020; Sugiyarto et al., 2023). Along with determining their 3D structures, we will determine TCR affinity, T cell avidity, pHLA stability and peptide affinity. These properties will complement 3D structures for understanding mechanisms of neoantigen discrimination and benchmarks for further therapeutic development.

Translational potential

This work has direct translational potential. Firstly, the neoantigens we're targeting are included in a cancer vaccine under development. TCRs and neoantigen peptide sequences themselves are modern drugs in the form of TCR-T therapy and vaccines, respectively. By determining their 3D structures and their biophysical properties, we can optimize the potential of these therapies via a rational, structure guided approach.

Training opportunities

Key techniques will include protein expression, protein refolding, protein purification, x-ray crystallography, structure determination, flow cytometry and sterile tissue culture. Biophysical techniques such as Biacore (surface plasmon resonance) will also be used. For some TCRs, their specificity may be interrogated via yeast display of large peptide lirbraries. Together the student will develop a suite a structural and functional assays to interrogate how the immune system detects public noeantigens. The Centre for Immuno-Oncology is a diverse group of immunologists and oncologists with a focus on exploiting fundamental immunological insights into novel cancer immunotherapies.

Rotational Project

T cells can eliminate tumours through detection of neoantigens presented on class I and class II human leukocyte antigens (HLA-I and HLA-II). Neoantigens derive from protein substitutions resulting from DNA base changes across the genome. Most neoantigens are 'private', derived from the unique complement of mutations found in an individual tumour. However, a subset of neoantigens are shared between cancers and patients across indications, due to common mutations in key oncogenic pathways. These 'public' neoantigens are derived from some of the most mutated genes in cancer including KRAS, p53 and EGFR. Critically, these neoantigens can be immunogenic and immunotherapies targeting these neoantigens have shown promising effects in early phase trials.

Along with partners, the Oxford Centre for Immuno-Oncology is developing an experimental cancer vaccine that targets 16 public neoantigens for use in patients at high risk of recurrence. It is critical that we understand as much as possible about how the immune system detects these 'public' neoantigens, to facilitate the development of this vaccine and other immunotherapies. As part of this effort, you will investigate the molecular basis for how 'public' neoantigens are presented by HLA-I and HLA-II molecules and detected by T cells. You will generate and purify multiple HLA-I and HLA-II proteins presenting 'public' neoantigens and TCRs suitable for x-ray crystallography trials. You will use x-ray crystallography to determine their x-ray crystal structures and determine their thermal stability. You will express TCRs in cell lines and primary T cells to determine their co-receptor dependency. Key techniques will include protein expression, protein refolding, protein purification, x-ray crystallography, structure determination, flow cytometry and sterile tissue culture. Biophysical techniques such as Biacore (surface plasmon resonance) will also be used. For some TCRs, their specificity may be interrogated via yeast display of large peptide libraries. Together the student will develop a suite a structural and functional assay to interrogate how the immune system detects public noeantigens.

Ideal student background: The student will have a first degree from a world class insitution in biochemistry, biomedical sciences, biological engineering or similar. Research experience in molecular biology and protein purification is desired but not necessary. The ideal candidate will want to make fundamental contributions to understanding immunology with the aim of improving cancer immunotherapy.

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41. 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 3 and Track 4 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.

Rotational Project: Injectable polymeric microparticles for the localised and sustained release of androgen receptor antagonists.

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 have shown significant survival benefit when AR antagonists are administered in the early stages of the disease. However, the use of AR antagonists is associated with the emergence of drug-resistant phenotypes and significant systemic side effects. The aim of this project is to develop locally injectable and biodegradable microparticles for the sustained and controlled release of commonly used androgen receptor antagonists. This approach aims to enhance therapeutic efficacy while reducing systemic side effects and the emergence of drug-resistant phenotypes compared to conventional administration methods.

Training opportunities
The student will mainly focus on the fabrication and characterisation of the proposed drug delivery system.
They will acquire skills in:
- Fabrication of drug loaded microparticles through microfluidic-assisted emulsion, using clinically approved AR antagonists and surface-eroding biodegradable polymers. Depending on the student's interest and expertise, they will have the opportunity to receive additional specialised training in polymer chemistry for the synthesis and fine-tuning of the polymers. They will be guided and supported as necessary by experienced chemists within the Stevens group.
- Characterisation of the drug loaded microparticles through different techniques, including but not limited to dynamic light scattering (DLS) and scanning electron microscopy (SEM).
- Degradation and release kinetic studies, based on both biochemical assays and single-particle Raman spectroscopy.
- Culture of prostate cancer cell lines.
- Initial validation of the microparticles for the effective delivery of AR antagonists using a prostate cancer cell line.

This 6-months rotational project will provide foundational knowledge and skills for the subsequent DPhil project, in particular for the optimisation of the drug loading and release profile for the localised, sustained and controlled release of AR antagonists at therapeutic levels over the desired period of time in pre-clinical prostate cancer models. Additionally, the skills acquired in handling prostate cancer cell lines and the knowledge gained in prostate cancer biology will be crucial for progressing to more complex in vitro and in vivo preclinical models to evaluate the efficacy, safety, and ability of the proposed delivery system to prevent the emergence of drug-resistant phenotypes.

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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42. 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 3 and Track 4 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.

Rotational Project: Using single-cell transcriptomics to understand PARP inhibitor resistance

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.

Training opportunities

The student will receive training in the analysis of large-scale data, in particular of single-cell RNAseq data, and related software such as R and related packages.

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.

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43. 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.

Rotational Project: Computational genomics approaches to error-prone polymerase mutagenesis

Carcinogens and mutagenic processes leave distinct footprints in DNA, detectable using the computational approach of *mutational signatures*[6]. 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 aim of this rotation project is to analyse somatic mutation data from large existing cancer patient databases and identify candidate mutational signatures of trans-lesion polymerases. Here, we will look at mutations in vicinity of regions where TLS polymerases get recruited (e.g., to bypass UV-induced damage). All the data is ready-to-use in the project. The results from this project can directly lead into the larger DPhil project.

Training Opportunities: The student will get training and practise in computational genomics, bioinformatics, large omics data integration, data visualization, statistics, and high-throughput computing. Support will be provided to develop soft skills in computational project design, critical thinking, writing, and presenting.

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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44. 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 3 and Track 4 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.

Rotational Project: Microsatellites and risk of cancer

Short tandem repeated sequences, or microsatellites, within DNA make up approximately 3% of the normal human genome and data suggests that they are involved in cellular processes and various diseases. However, we know very little about the role of microsatellites in cancer risk. The aim of this project is to assess the role of inherited variation in microsatellites in increasing the risk of colorectal cancer using whole genome sequencing data from the UK Biobank and 100,000 Genomes Project. The 6-month project would involve the development of relatively simple methods to extract microsatellite genotypes, gene expression and other molecular data from large repositories, such as 100,000 Genomes, and in-house data. Colorectal normal and tumour tissue will be the focus. Initial analyses would comprise microsatellites previously reported as associated with differences in gene expression in other tissues, but would be extended to other microsatellites should time permit. Microsatellites that predispose to colorectal cancer will be identified based on allele frequencies in colorectal cancer cases and population controls. This work would form a distinct project in itself or lead naturally into a full DPhil project.

Training opportunities: The student will receive training in manipulation and analysis of large molecular and clinical. Data sets, including whole-genome sequencing data, such as those from the UK Biobank, in statistics and bioinformatics, and relevant software for analysis. Training in developing simple code will be provided if required.

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

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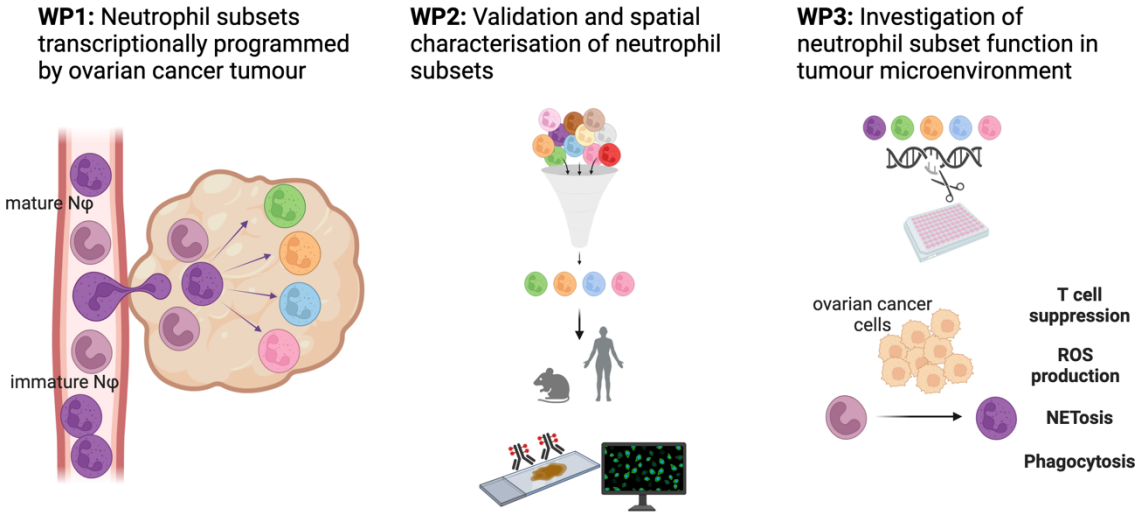
45. Spatiotemporal heterogeneity of neutrophil subsets in ovarian cancer – Irina Udalova

Primary Supervisor: Irina Udalova
Additional Supervisors: Sarah Spear
Eligibility: Track 3 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 in basic immunology techniques like flow cytometry, RT-qPCR and in vitro cultures to analyse the outcomes. Furthermore, insights and potential guided analysis of single-cell RNA sequencing as well as cutting-edge microscopy and spatial transcriptomics (GeoMx and CosMx Nanostring platforms) to define the localisation of myeloid cell subsets within the tumour microenvironment will be made available.

Rotational Project: Spatial organisation of myeloid cells in murine and human ovarian tumours

Our preliminary data demonstrate that neutrophils infiltrate the omentum early post tumour cell seeding, following monocyte-derived macrophages. We hypothesise distinct neutrophil subpopulations will localise to different areas of the tumour microenvironment (TME), and this influences which molecular pathways are expressed. We propose that the high-grade serous carcinoma (HGSC) TME reprogrammes myeloid cells into specific subsets that support tumour growth. Murine tumour sections and human tissue microarrays (already established by our collaborator) will be investigated for the geographical location of myeloid cell subsets using the developed imaging approaches and, where appropriate, innovatve genetic mouse strains, with fluorescent markers labelling myeloid cells. Well-validated antibodies available will be selected to visualise neutrophil subsets. The distance between neutrophils and neighbouring cells will be assessed. We will utilise the multiplex immunofluorescence platforms.

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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46. 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 3 and Track 4 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 multidciplinary teams to improve efficiency in conducting this research.

Rotational Project: Investigating the association between blood test trend and cancer risk

Clinical guidelines for primary care include single blood test abnormalities to identify patients with increased risk of undiagnosed cancer. Blood test changes over time may improve cancer risk stratification by considering a patient's individual baseline and identifying important changes within the normal range. The goal of this project would be to assess whether blood test trend is associated with cancer risk.
Data is available from the Clinical Practice Research Datalink primary care database, which is linked to other databases. The candidate will use smoothing techniques to produce graphs of trends over time by cancer presence (yes/no) and utilise dynamic modelling techniques to assess the association between repreatedly measured blood tests and cancer risk. Diagnostic accuracy of trend will be assessed, including the area under the curve, sensitivity, specificity, and positive and negative predictive value of trend. This project will build an evidence base for the role of blood test trend for cancer detection.

Training opportunities: The candidate will develop experience in data management and analysis of large electronic health records data, dynamic modelling, and academic writing. The candidate will also have the opportunity to network with our multidisciplinary Cancer Theme to learn of other ongoing research and build collaborations.

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47. Novel forms of cell-cell communication in cancer – Richard White

Primary Supervisor: Richard White
Additional Supervisors: Yang Shi
Eligibility: Track 3 and Track 4 applicants are eligible to apply for this project

Abstract
Tumor cells exist in a complex ecosystem with numerous other cells in the microenvironment (TME). How these cells communicate with each other to enable tumor initiation and progression remains poorly understand. To address this requires experimental systems that encompass the entire cellular complexity of the TME. Towards this end, we have developed the zebrafish as a new model for studying tumor-TME crosstalk (White *Nature* 2011).

Why zebrafish? The major advantages of using fish for these studies is that each relevant cell type can be easily genetically manipulated using CRISPR and related approaches. Moreover, fish are optically transparent, which allows us to image cancer from the very first cell onward. This combination of strengths allows us to interrogate the entire complexity of the cellular ecosystem in cancer (Weiss *eLife* 2022).

Previous work from the lab has established a zebrafish model of melanoma in which the BRAFV600E gene is expressed in the melanocyte lineage. These animals develop melanoma that closely resembles the human tumor (White *Nature* 2011). We have used this model to understand the mechanisms by which BRAF transforms melanocytes. These mechanisms include the epigenetic state of the melanocyte (Baggiolini *Science* 2021) and the anatomic position of the melanocyte (Weiss *Nature* 2022). More recently, we found that the transforming ability of BRAF is tightly dependent on cells within the TME. We discovered that the newly transformed melanocytes are in direct communication with another nearby cell called the keratinocyte. These two cells form electrical connections, and signal through GABAergic receptors (Tagore *Cancer Discovery* 2023). Subsequent work has shown that when melanocytes and keratinocyte interact with each other, they each undergo epigenetic remodeling. Analysis of these cells either alone or together (ATAC-seq, ChIP-seq and RNA-seq) revealed specific changes that only occur when the cells are in contact with each other.

Research objectives and proposed outcomes
In this D. Phil project, our aim is to understand the epigenetic proteins that mediate reprogramming of these cells. In Preliminary Data, we have performed a screen against a large panel of epigenetic proteins, and found several promising hits that appear to mediate these effects. Based on this data, the specific goals of the project include:

- Perform genetic overexpression and knockout studies of candidate epigenetic proteins in both melanocytes and keratinocytes
- Assess how these genetic perturbations affects melanoma initiation and progression in the zebrafish model
- Identify the biochemical mechanisms by which the identified epigenetic protein affects target gene expression
- Assess the extent to which these mechanisms are conserved in human melanoma
- This work will be facilitated by the co-supervisor Prof. Yang Shi, a world expert in epigenetics

Translational potential of the project.
Understanding the basic mechanisms by which tumor cells communicate with the TME is of direct relevance to human cancer biology. Epigenetic proteins identified in our analysis could be used to prevent melanoma.

Training opportunities

The student will gain expertise in cancer biology, tumor microenvironment, zebrafish genetics, human cell culture, epigenetic profiling, informatics and single-cell biology

Rotational Project: Genetic validation of epigenetic candidates

For a rotation project, the student will create genetic knockouts of all of the hits we identified in our initial screen of melanoma/keratinocyte interactions. This will be done with CRISPR. The student will then perform basic phenotypic analyses both in vitro using cell lines as well as in vivo using zebrafish. This data would then form part of their eventual thesis work, as these knockouts are an essential part of downstream mechanistic analyses. They would be carefully mentored by others in the lab to learn all of these essential genetic techniques.

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

Primary Supervisor: David Withers
Additional Supervisors: Audrey Gerard
Eligibility: Track 3 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.

Rotational Project: How does immune supportive stroma within the tumour microenvironment alter dendritic cell fate?

Dendritic cells (DCs) lie at the heart of anti-tumour immunity, controlling the magnitude and fitness of the adaptive immune response through cellular crosstalk within both the tumour and draining lymphoid tissue. Despite appreciation of the critical role played by DCs, precise understanding of how DC behaviour, fate and function are regulated within the tumour microenvironment remains limited. In lymphoid tissues (e.g. lymph nodes), the behaviour of DCs is intimately tied to specialised fibroblast populations that create specific microenvironments. Thus, optimal DC functions require discrete cellular niches. Within tumours, a spectrum of organised aggregates of immune cells (collectively termed 'tertiary lymphoid structures' or TLS) can form and are thought to require similar support from specialised fibroblasts. Crucially, TLS within or near tumours are associated with a significantly better prognosis in multiple cancer types, indicating that these aggregates improve anti-tumour immunity. This suggests that TLS impact local DC functions. The mechanisms influencing TLS formation remain poorly defined as do the specific immune functions different TLS support.
This project will investigate how the presence of specific fibroblast populations alters intratumoural DCs, utilising established *in vivo* models that allow the addition of different fibroblast populations to tumours. Here we will specifically assess the intratumoural DC compartment in the presence of immune supportive fibroblasts. Our preliminary data indicates that the addition of these fibroblasts enhances TLS formation. To develop novel insight into how these structures, change DC behaviour, we will exploit photo-labelling techniques pioneered in the Withers lab, enabling specific testing of DC recruitment, retention and egress using flow cytometry. Immunofluorescence imaging of tumours will provide further insight into how the location and organisation of these cells is changed in the presence of the fibroblasts. Our working hypothesis is that the introduction of immune supportive fibroblasts into the tumour microenvironment, supports sustained DC functions resulting in enhanced CD8 T cell responses and improved tumour control.

This project will develop understanding of the immune cell composition of the tumour microenvironment, the cellular interactions that shapes this and experimental approaches that can be used to interrogate this fascinating area of immunology.

Summarise the training opportunities
This 6 month project will provide comprehensive training in core cancer immunology skills. Specifically, these include *in vitro* culture and maintenance of cancer cells and other cell types (e.g. fibroblasts), isolation of cells from mouse tissues, detailed flow cytometric analyses to characterise immune cells and confocal imaging approaches to localise immune cells and explore tissue niches. Importantly, this project will build experience in the use of *in vivo* cancer models, essential for interrogating the anti-tumour immune response in depth. Furthermore, training in how to use cutting-edge experimental approaches including photo-labelling will be provided, building understanding of how genetically modified mouse models can be used to interrogate immune cell behaviour and function. Given the timeframe of this project, the *in vivo* experimentation will be performed by existing lab members and tissues samples brought back to the lab for analysis.

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

Primary Supervisor: Nancy Zaarour
Additional Supervisors: Ahmed Ahmed
Eligibility: Track 3 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

Rotational Project: Optimising an *in vitro* assay to evaluate the immunogenicity of ovarian cancer specific peptides

Despite many advances in therapeutics, the survival rates of HGSOC have only modestly improved. More than 80% of ovarian cancer patients experience recurrence, and more than a half of these patients die in less than five years post-diagnosis, emphasizing a substantial need for the development of strategies that have the potential to prevent tumor development. Our preclinical model created a foundation for developing a prophylactic vaccine to prevent ovarian cancer. Building on our recent discovery demonstrating that specialised T cell populations in the FTE, site of origin of HGSOC, are capable of recognising metastasis-derived cancer cells, in concert with the identification of tumor-derived peptides that arise at the earliest stage of cancer development, we propose to test the hypothesis that a vaccine that we designed targeting ovarian cancer tumour associated antigens (TAAs) and recurrent hotspot mutations could be effective in eradicating early transformed cells in the FT. This work will involve optimising in vitro assays to evaluate the immunogenicity of our peptides library, priming *de novo* immunity and memory recall in patients/and healthy individuals blood. T cells isolated from PBMCs, fallopian tubes and tumour infiltrating lymphocytes (TILs) will be interrogated for their ability to respond to our selected peptides. Moreover, *in vitro* primed naïve T cells or induced memory cells will be tested for their ability to induce cytotoxic effect to autologous ovarian cancer organoids and early-transformed fallopian tube epithelial cells.

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.

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50. GraphECD: Developing Graph artificial intelligence solutions for Early Cancer Detection with primary care electronic health records data – Tingting Zhu

Primary Supervisor: Tingting Zhu
Additional Supervisors: Yu Liu, Pradeep Virdee and Brian Nicholson
Eligibility: Track 3 and Track 4 applicants are eligible to apply for this project

Abstract
Cancer, the second leading cause of death globally, significantly impacts individual health and overwhelms healthcare systems. Despite recent advancements, early cancer detection remains a major challenge due to the diverse types of cancer and their complex interactions. Routine systematic health checks are impractical for both individuals and healthcare systems. Thus, the most frequently requested blood tests in NHS General Practice serve as a crucial resource for early cancer detection. Despite extensive electronic health records (EHRs) have been accumulated over the years, individual GP blood tests are sparse due to infrequent visits and varied test prescriptions based on symptoms. This research aims to harness the power of graph artificial intelligence (AI) to analyse and utilize extensive data for enhancing early cancer detection. By organizing patients and biomarkers into graph networks, we aim to capture relationships and identify primary factors for different cancer types, providing both effective and interpretable results for clinical application.

Research objectives and proposed outcomes
Background: Currently, only about 54% of cancers are diagnosed at an early stage, which is significantly below NHS England's target of 75% early cancer detection by 2028 [1]. While AI advancements have introduced early cancer detection methods [2, 3], most studies focus on imaging and genetic data, largely neglecting the commonly collected blood test data. Given the digitalization of healthcare systems, extensive EHR data, encompassing millions of blood test results over several years, stands out as a promising source for AI-based early cancer detection [4, 5]. The complex relationships between blood biomarkers and cancer types present a significant challenge, i.e., blood biomarkers can indicate a range of conditions and their interactions can be subtle and multifaceted, necessitating sophisticated and interpretable AI solutions. Traditional AI models may struggle with the intricacy of these relationships, leading to the need for more advanced, interpretable AI techniques, such as graph neural networks, which can model the relational structure of data, making them well-suited for capturing the nuanced interactions between biomarkers and cancer.

Aim: To build graph AI models with blood tests from primary care for early cancer detection.

Objectives: There are three main objectives:
1) *build and analyse graph networks of blood biomarkers and patients* – the student will learn of network construction and analysis techniques, such as community detection, to detect similar cohorts in patients and as well as biomarkers with similar functions, e.g., sodium, potassium and creatinine for renal function. These constructed networks will capture the relatedness among patients and biomarkers through relational edges. The student will apply advanced graph analysis techniques to extract meaningful patterns and insights, such as node centrality, clustering coefficients, and shortest path analysis, to understand the network's topology and connectivity. Supervisors will guide on data curation and understanding of network analysis results.

2) *develop and evaluate graph artificial intelligence models for early cancer detection* – the student will learn of representation learning and develop graph neural network-based models with the constructed graph networks of patients and biomarkers for early cancer detection. The complicated interactions between blood

biomarkers and cancer types will be modelled with message passing mechanisms in respect to patient-specific subgraphs, and final model performance will be evaluated with different cancer types. The development process will include rigorous training and validation of the models using diverse datasets to ensure robustness and accuracy.

3) *identify and explain highly related blood biomarkers for different cancer types* – the student will learn of interpretable machine and deep learning techniques such as SHAP (SHapley Additive exPlanations) and attention mechanisms. These techniques will help elucidate the relationships between various blood biomarkers and cancer types, enabling the identification of key indicator biomarkers for specific cancers. The student will analyse how these biomarkers' levels change based on personal, clinical, and cancer characteristics, such as age, sex, comorbidities, diagnosis routes, cancer site, and stage. This analysis will provide a comprehensive understanding of biomarker trends and their implications in early cancer detection.

Data: Data from ~30 million patients from the CPRD primary care database is available to develop the models. It includes information on patient characteristics, 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 graph-based prediction models that leverage graph neural network for early cancer detection with interpretable results provided. Outputs will include peer-reviewed journal publications for each objective separately and conference presentations. This project will support cross disciplinary collaborations between primary care and engineering, bring together experts in big data, machine learning, oncology and qualitative research.

This research will develop graph artificial intelligence solutions with blood test data for early cancer detection and inform clinical practice. The DPhil candidate will develop leadership and research skills in various areas, including technology/tool development, 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.

Translational potential of the project

This project is one of the first attempts to introduce graph artificial intelligence with primary care data for early cancer detection. It will build graph networks from blood biomarkers and patients, which provide insightful modelling to the research area, and discover significant patterns between blood biomarkers and cancer types for clinical reference. The outputs of this work will enable a new approach to detect cancers within an early stage, minimising psychological and physical harm to patients and economic costs of unnecessary testing in the NHS. A key feature of this project is the interpretable mechanism design, which ensures that the AI solutions are transparent and trustworthy. This transparency is crucial for practical implementation, as it allows patients, clinicians, and service managers to understand and respond to the results effectively. The project's outcomes will not only advance the scientific understanding of the relationship between blood biomarkers and cancer but also inform the refinement of prediction tools. These advancements will support the development of software implementations and guide larger prospective evaluations, ultimately enhancing the overall effectiveness and efficiency of early cancer detection systems.

Summary of training opportunities

Throughout this DPhil, skills and experience will be developed in conducting independent research, working with routinely collected linked electronic health records data, patient and public involvement, statistical

analysis, deep learning techniques, general skill development, and more. Attendance and presentation of findings at scientific conferences will also be encouraged. The student will be offered a comprehensive training programme and encouraged to attend relevant courses. There will be the unique opportunity to be trained in use of CPRD (the one of largest primary care databases in the UK linked to cancer, mortality and hospital records) and qualitative methodology expertise of both the Department of Engineering Science and the Department of Primary Care Health Sciences. Overall, this DPhil will provide a multidisciplinary training experience, preparing the student to become a leader in the intersection of AI, healthcare data, and cancer research.

Rotational Project

Simple blood tests play an essential role in identifying patients for cancer investigation. The current evidence base is limited almost entirely to tests used in isolation. However, recent evidence suggests combining multiple types of blood tests and investigating trends in blood test results over time could be more useful in selecting patients for further cancer investigation. Such trends could increase cancer yield and reduce unnecessary referrals. The BLOod Test Trend for cancEr Detection (BLOTTED) project aims to develop clinical prediction models that incorporate trends in blood tests to identify cancer risks.

This short-term rotation project focuses on developing machine learning methods for predicting cancer risks in patients. To this end, this project seeks to include a study on the following:

(1) To provide descriptive statistics for describing patterns in blood biomarkers, such as the frequency and timing of tests, and summarise the results of each blood test, overall and stratified by personal characteristics and cancer types. Associations between patient characteristics and cancer types in blood testing will be examined.
(2) To construct a patient-biomarker network for capturing the relatedness among patients and biomarkers, such as the frequently-tested biomarkers to patients, and highly similar patients in the clinical records. Community detection techniques will be applied to find meaningful patient clusters.
(3) Explore methods in graph neural networks for early cancer detection and compare it with traditional machine learning methods such as Random Forest and Support Vector Machine. Investigations of different biomarker's influence on modelling.

This project may act as a foundation for a DPhil, which will focus on (i) building and analysing graph networks of blood biomarkers and patients, utilising the rich heterogeneous and longitudinal medical data that are commonly available in primary care; (ii) developing and evaluating graph artificial intelligence models for early cancer detection; and (iii) identifying and explaining relevant blood biomarkers for different cancer types to provide cancer risk prognosis, and particularly those which are rare with limited patient data.

Data availability: the accepted candidate will be able to access the Clinical Practice Research Datalink (CPRD https://www.cprd.com/). The CPRD is an observational database of electronic general practitioner health records for over 35 million patient lives, including 10 million currently registered patients. For this project, the data covers everything that is coded in primary care patient records between 2000 and 2019 including demographics, diagnoses, clinical measures, test results, immunisations, and prescriptions. It can be linked with other data resources, such as the Office for National Statistics Mortality Data, Hospital Episodes Statistics and various UK registries, such as the Cancer Registry.

Ideal student background: The doctoral candidate shall have a background in machine learning, biomedical informatics, health data science, epidemiology, computational biology, or a related discipline is required. Prior experience in handling large healthcare datasets, 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 translating data-driven solutions into clinical settings. 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.

References

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[4] Fitzgerald, R.C., et al. 2022. The future of early cancer detection. *Nature Medicine*, 28(4), pp.666-677.
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