DPhil in Cancer Science

University of Oxford

Clinical

2026 Intake Project Booklet

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DPhil in Cancer Science – Clinical/ Medical Undergraduate Project Booklet

Introduction

This handbook provides an overview for prospective students looking to study for a DPhil in Cancer Science starting in 2026 at Oxford University. The Programme provides research based doctoral training for cancer researchers from clinical, biological, engineering, mathematics, and statistics background. Students will receive a world-leading research training experience that integrates an education initiative spanning cancer patient care, tumour biology and research impact; on- and post-programme mentorship; and a specialised, fundamental, subject-specific training tailored to individual research needs. Students participating in the scheme will be offered:

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

At the end of their DPhil course, students should:

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

Selection Criteria & Eligibility

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

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

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

Application Track 2 – Medical Undergraduates. Medical students who are currently undertaking a primary medical qualification (MBBS, MBChB or equivalent). At entry, we will be looking for evidence of completion of at least the first two years of a primary medical qualification and achievement at the level of an upper-second or first-class honours degrees (or iBSc).

All applicants will be judged on the following:

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

Funding

All offered places are fully funded at the home rate. This includes salary/stipend, University/College fees, and a research consumables budget.

Salary and stipend provisions are summarised below:

- **Application Track 1:** 3 years of salary at Grade E63 or E64 Clinical Researcher rate.
- **Application Track 2:** 3 years of stipend at the flat rate of £22,123 per annum.

Applications from international students (including EU) will be accepted, however the funding from CRUK only covers fees at the home rate, so successful international candidates will be

awarded top-up funding from elsewhere in the institution to cover the remaining fees. For more information about funding please see Funding — University of Oxford, Medical Sciences Division.

International students are also welcome to apply for external scholarships,

Notable Scholarships

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

How to Apply

A detailed summary on how to apply can be found here. In brief, prospective students **must** apply with a **prioritised list of three projects selected from this booklet by midday on Tuesday 2nd December 2026.** Shortlisted students will be invited to interview in January. It is strongly suggested that students contact supervisors of projects they are interested in applying for prior to application.

Projects

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

Contents

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

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

Abstract of the project

To protect the genome from damage organisms have evolved a cellular defence mechanism termed the DNA damage response (DDR). The DDR includes a diverse set of signal transduction pathways and effector proteins that act to sense DNA lesions and effectively repair the damage, limiting the propagation of genomic instability. Exploiting DDR pathways to specifically target and kill cancer cells has become an attractive therapeutic avenue within cancer research. This is exemplified by the synthetic lethal interaction between PARP inhibition and *BRCA1* or *BRCA2*-deficient tumours[1]. Our 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 specific and efficient modification of target proteins by PARP1 [3,4]. Crucially, our work also identified ARH3 and several other hydrolases which specifically remove PARP1-dependent ADP-ribosylation[5,6]. Taken together, the insights on regulation of DNA damage inducible 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 knockouts of ADP-ribosylhydrolases or their overexpression in model cell lines associates with PARP inhibitor (PARPi) resistance or sensitivity[7]. Based on these results, we hypothesize that ADP-ribosylhydrolase 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 ADP-ribosylhydrolase 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 ADP-ribosylhydrolase expression cells to PARPi is unknown, and elucidating this mechanism will be a major goal of this proposed work.

Research objectives and proposed outcomes

Objective 1. Characterise the effect of ADP-ribosylhydrolases (ARH3, PARG, MACROD2..) under- and overexpression in a series of model cancer cell lines on PARP inhibitor sensitivity/resistance. We will collect and test a variety of ovarian cancer cell lines, profiling them for ADP-ribosylhydrolase protein expression levels and then treating with several different PARPi of varying PARP-trapping capabilities (olaparib, talazoparib, veliparib). To determine the impact of levels of ADP-ribosylhydrolases 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 ADP-ribosylhydrolase levels by knockdown, knock out and inducible overexpression in a our standard model U2OS cell line as well as 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 ADP-ribosylhydrolases will assess the suitability of these enzymes as targets for the development of inhibitors.

Objective 2. Elucidating the mechanistic basis for the sensitivity/resistance of cells with deregulated ADP-ribosylhydrolase expression cells to PARPi (modulation of the PARP-trapping, regulation of DNA repair pathway choice, regulation of the chromatin structure/epigenetic marks, effect on cell differentiation and apoptosis). For these studies we will use largely cell biology/biochemical and genomics approaches.

Objective 3. To determine the frequency of ADP-ribosylhydrolase genes 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, MACROD2, PARP1 and ADPr marks, by immunohistochemistry (IHC) on two independent sets of tissue microarrays (TMAs) containing a total of 1200 ovarian cancers (obtained from Prof Ahmed Ahmed laboratory at the Nuffield Department of Women's & Reproductive Health, University of Oxford).

Translational potential of the project

Our data suggest that ADP-ribosylhydrolase 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 ADP-ribosylhydrolase proteins, 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 and immunohistochemistry methods.

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

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

Primary Supervisor: Hagan Bayley
Additional Supervisors: Mara Artibani and Dr Linna Zhou
Eligibility: Track 1 and 2 applicants are eligible to apply for this project

Abstract of the project
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 survives 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 hematological 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, Dr Artibani 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 and Dr Linna Zhou 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**).[6] 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)**.

c) Research objectives and proposed outcome

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. 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 ECM materials, including synthetic peptide gels, 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. With proven expertise in their respective fields, the supervisors will provide critical guidance and support to the candidate throughout the project.

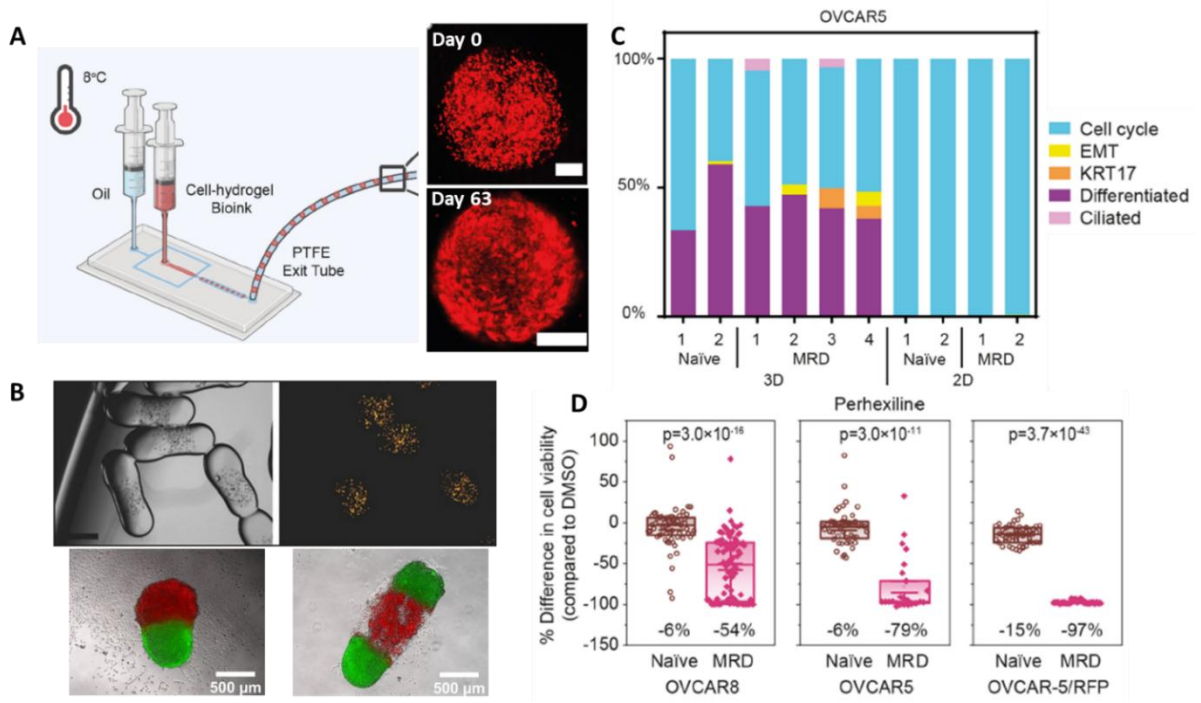

Figure 1: Microfluidic-based 3D microtumour technique and drug testing using the 3D MRD microtumours.[6] **A**, Schematic illustration of 3D microtumour fabrication by the microfluidic platform (left) and long-term culture of 3D microtumours composed of OVCAR-5/RFP (red) and Matrigel (right). Scale bars 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 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 cause of cancer deaths. The proposed project would combine a novel microfluidic technique and patient-derived cells to fabricate realistic 3D MRD models that are hard to achieve with the organoid method. Specifically, we will advance the previously established 3D MRD microtumour platform by

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

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

Ideal student background: The proposed project is multidisciplinary. The potential candidate will have a background in either cancer biology, bioengineering (or another engineering field), or biochemistry. Experience in cell and organoid culture is desirable. Experience in microfluidics, hydrogels and biofabrication is favorable, 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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3. Leveraging systemic disturbances to amino acid metabolism to interrogate the impact of histone modifications on cancer development and host defences – Pablo F. Céspedes

Primary Supervisor: Pablo F. Céspedes
Additional Supervisors: Pawel Swietach
Eligibility: Track 1 and 2 applicants are eligible to apply for this project

Abstract of the project
As demonstrated by multiple epidemiological studies, oncogenesis and cancer progression are influenced by risk factors such as dysregulated metabolism and immune responses. However, our understanding of cancer biology has been informed by a combination of *in vitro* experiments using standardised media that inadequately model the tumour microenvironment and *in vivo* studies that have limited scope for controlling metabolic variables. One strategy for interrogating the influence of metabolic disturbances on immune cell functions is using genetically altered mice with systemically disturbed metabolism. Such animal models can produce sustained metabolic disturbances of magnitude and duration necessary for tracking immune responses in a complete organism.

In this project, you will study the consequences of disturbed amino acid metabolism in a mouse model of impaired processing of propiogenic substrates. These substrates, including isoleucine, valine and methionine, are catabolised to propionyl-CoA, an ester of three-carbon propionate. Normally, mitochondrial propionyl-CoA carboxylase (PCC) converts this intermediate to a four-carbon derivative that can enter the Krebs cycle. However, loss-of-function mutations in PCC produce a build-up of propiogenic substrates, which clinically manifests as the disease propionic acidaemia. From an experimental viewpoint, mice with impaired PCC activity generate a milieu that is enriched in the propionylating agent propionyl-CoA, the histone deacetylase inhibitor propionate, and the methylating agent S-adenosyl-L-methionine (SAMe). We have shown that these mice experience a systemic change in histone methylation and acylation, offering unprecedented insight into the consequences of these epigenetic changes on the host organism, developing tumours, and immune responses. You will use these mice to obtain biological materials for detailed studies using state-of-the-art methods. We will determine the impact of sustained histone modifications and describe mechanisms for the interplay between metabolic disturbances, immune surveillance, and cancer progression. This effort will define and test the scope of metabolic interventions in modulating cancer risk and progression. To support you in this ambitious and innovative project, we offer combined expertise in immunology and metabolism, and a bespoke programme of interdisciplinary training and mentorship.

Research objectives and proposed outcomes
Aim 1: To elucidate the regulatory roles of propionyl-CoA and SAMe accumulation on the differentiation of T cells into memory subsets and their anti-tumor effectors. We have developed innovative tools for you to study the effects of histone modifications on epigenetic, structural and functional adaptation of T cells to homeostatic environments and the tumor milieu. The synergy between the Cespedes and Swietach labs will expedite your efforts to elucidate the roles of these metabolic intermediates in T-cell differentiation in otherwise healthy human organoids and mice, and test their killing efficacy of xenografted tumors.

Aim 2: To elucidate how altered propionyl-CoA and SAMe fluxes influence tumor growth, immune resilience and spread. You will identify the cell-intrinsic effects of disturbed amino acid metabolism in terms of anti-tumor immunity at a T-cell centric level, influence on tumor growth, immune resilience and metastasis. The project will offer opportunities to characterise the tumor milieu using spatial transcriptomics and high-dimensional flow cytometry. Using our bespoke lymphoid organoids and tumoroid methods, you will assess whether the overload

of propiogenic substrates leads to changes to the dynamics of human T-cell cytotoxicity and its correlation with biochemical, cellular, and physical aspects of the tumor milieu, including pH.

Translational potential of the project

As the project matures, you will develop *ex vivo* models for wider translational immuno-oncology and experimental medicine studies. Translation of your observations made in the animal model to CRISPR/Cas9-edited cells will deliver timely societal and scientific impact and much-needed mechanisms. The concurrent development of bespoke human *ex vivo* systems will reduce animal experimentation as part of our commitment to the 3Rs. A realistic translational opportunity is to repurpose precision diets for managing systemic metabolic disturbances for use in controlling tumour biology and immune responses. This effort leverages of a wealth of knowledge obtained from managing various metabolic disorders in patients.

Training opportunities

You will work closely with an inter-disciplinary team to elucidate whether, and how, T cells differentiate across gradients of propiogenic substrates using the mouse model as well as *ex vivo* models of human immunity. A programme of research, tailored to your interests, will involve high-dimensional flow cytometry (conventional and spectral), high-content microscopy, proteomics, metabolomics, and conventional techniques across molecular and cellular immunology and physiology. The generation of OMICS datasets and the use of genetic and pharmacological manipulations will be applied to human *ex vivo* systems, including lymphoid organoids and histocultures (explants), for a complete and ambitious project.

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4. Design of novel CD4+ T cell platforms for chimeric antigen receptor-T cell therapy – Ronjon Chakraverty

Primary Supervisor: Ronjon Chakraverty
Additional Supervisors: Persephone Borrow, Paresh Vyas
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract of the project

The recent finding that B-lineage blood cancers can respond to anti-CD19 chimeric antigen receptors (CAR) T cells is a major clinical advance. However, treatment failure is observed in 50% or more of patients (1), arising most commonly through the escape of immune edited variants or failure of the T cell response. Accordingly, most technological advances in the field are focussing upon broadening T cell reactivity to multiple antigens or improving the function and survival of transferred T cells. However, this innovation has mostly centred around role of CD8+ T cells, while the role of CD4+ T cells in promoting anti-tumour immunity is under-explored. In this project, we will address how CAR transduction of CD4+ T cells with distinct cellular states will shape an anti-CD19 CAR-T response in vivo. We will compare CD4+ T cell populations in terms of their capacity to contribute to direct tumour control versus providing help to co-infused CD8+ CAR-T cells, as well as examining their broader engagement with host immunity and long-term fate. This information will be used in the design of novel CD4+ T cell platforms for antigen-specific adoptive T cell therapy.

Research objectives and proposed outcomes

Background: Preclinical studies have shown that CD4+ T cells are important players in promoting anti-tumour immunity(2). As a function of their plasticity, CD4+ T cell populations exert anti-tumour responses through a broad array of mechanisms including cytotoxicity, the provision of help for B cells or CD8+ T cells, or the wider engagement of host immunity. Two distinct cellular states, that of cytotoxic CD4+ T cells (Tctx) and follicular helper CD4+ T cells (Tfh) are of interest because they generate robust anti-tumour immunity via distinct mechanisms (cytotoxicity versus T/B help, respectively) (3, 4) and because their differentiation is reciprocally regulated and mutually antagonistic(5, 6). Thus, Tfh and Tctx responses often do not occur within the same experimental models or same locality, making direct comparisons of anti-tumour proficiency difficult. While CD4+ T cells are components of CAR-T products and promote efficacy, the precise mechanisms involved and the CD4+ T cellular states that contribute most to anti-tumour immunity are less clear. This lack of information is important because most engineering strategies to improve CAR-T efficacy (e.g., using small molecules, cytokines or genetic re-wiring) have been directed at modifying CD8+ T cells but do not address how they will affect the CD4+ T cell component of the product. To address this question, we have developed methods to isolate human circulating PD-1+CXCR5+ Tfh and SLAMF7+ Tctx cells by flow cytometry (Fig. 1A-B) and expand these populations by modifying rapid expansion protocols used for CAR-T manufacture (Tfh, Activin A, IL-12. IL-7; Tcx, high dose IL-2); under these conditions, each subset retains their canonical profile as assessed by flow cytometry and transcription for up to 21 days in culture (Fig. 1C). When co-cultured with autologous CD8+ T cells, we have found that Tfh cells shifted CD8+ T cell differentiation towards a 'less differentiated' phenotype with transcriptional profiles of memory T cells, whereas Tctx had little effect (Figure 1D-G). Our proof-of-concept that we can isolate and expand each human CD4+ T cell population now provides an important opportunity to directly compare their functions.

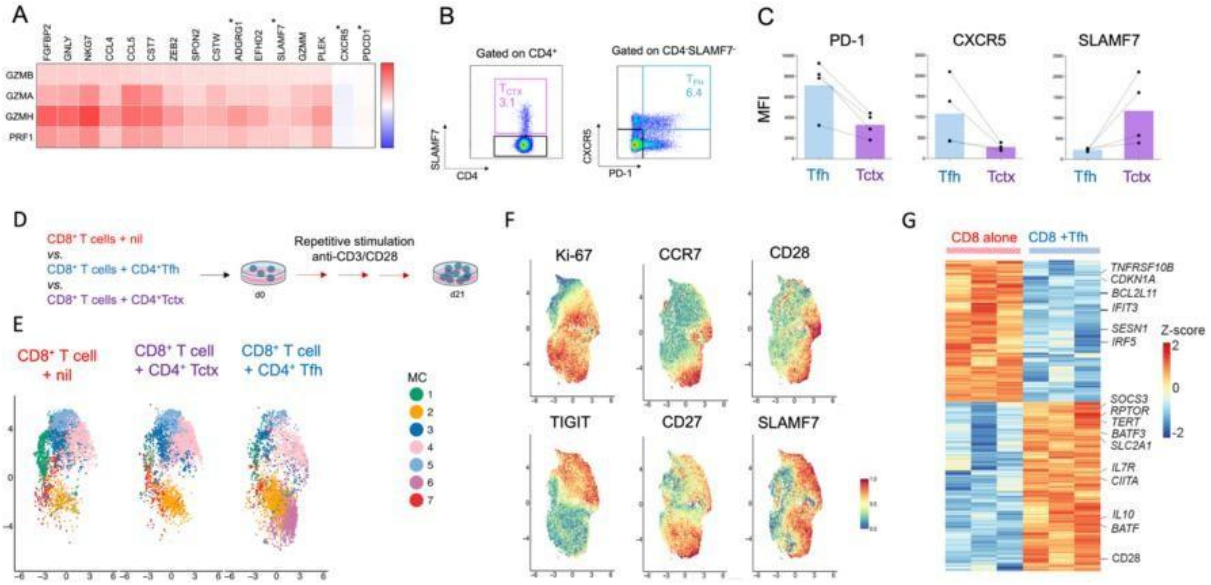

Figure 1: *CD4+ Tfh and CD4+ Tctx selection and expansion ± co-culture with CD8+ T cells. A Gene correlation matrix showing correlation of cytotoxicity genes with SLAM7. B Flow cytometric plots showing markers used to sort CD4+CXCR5+PD-1+ Tfh-like cells or CD4+SLAMF7+ Tctx. C. After 21 days culture, Tfh and Tctx maintained their phenotype. D. Tfh and Tctx were co-cultured with CD8+ T cells using a rapid expansion protocol over 21 days. E. UMAPs showing clustering of CD8+ T cells derived from a 23-marker flow panel. F. Projections of individual markers are shown. G. Heat map showing CD8+ T cell up-regulation of genes linked to memory formation and down regulation of genes linked to exhaustion and senescence following co-culture with Tfh after 21 days.*

1. What are the direct anti-tumour functions of CD4+ CAR-Tfh or CD4+ CAR-Tctx? As a 6-month project or part of a DPhil project, we will generate CD4+ CAR-T by selecting Tfh and Tctx before electroporation/RNP based CRISPR gene editing of the TRAC locus to knock down the endogenous TCR to prevent alloreactivity versus 3rd party tumour or xenogeneic GVHD. Flow sorted TCRneg cells will then be transduced with lentivirus containing an anti-CD19 CAR construct that incorporates a CD34 mimotope to allow immune-magnetic selection of transduced T cells. CD4+ CAR-Tfh or CD4+ CAR-Tctx will be co-incubated with CD19-expressing human B-lineage ALL cell lines in vitro (e.g., Nalm-6) and analysed for effector cytokine generation, activation marker expression and killing function; these will be compared to a CD8+ CAR-T (positive control) and mock transduced CD4+ Tfh or Tctx cells (negative controls). We will then compare CAR sensitivity of each CD4+ T cell subset (and in relation to CD8+ T cells) using the Nalm-6 CombiCell model(7); this experimental system, designed by Dushek group, allows precise titration of CD19 surface expression as well determination how accessory molecules (e.g. LFA-1) influence CAR sensitivity. As part of a continuing DPhil project, we will then test the respective capacity of each individual CD4+ CAR-T subset for direct control tumour control in vivo by infusing into NSG recipient mice engrafted with Nalm-6 using systems already set up in the laboratory. We anticipate that CAR-Tctx will demonstrate greater direct control of Nalm6 growth than CAR-Tfh.

2. Do CD4+ CAR-Tfh or CD4+ CAR-Tctx provide help to co-transferred CD8+ CAR-T cells? Using the same in vivo model described above, we will adoptively transfer a sub-optimal dose of CD8+ CAR-T (that partially controls Nalm-6 growth) either alone or in combination with CD4+ CAR-Tfh or CD4+ CAR-Tctx. CD4+ T cell helper functions for CD8+ T cells will be evident where the level of tumour control following co-transfer of the CD8+ and CD4+ CAR-T cell subsets exceeds the sum of tumour control when each subset is transferred alone. We anticipate that Tfh will demonstrate greater helper functions that Tctx. If a helper effect is observed, this would provide an opportunity to define the mechanism for help and we will be guided by data from recent CD8 Tfh interactome experiments which have yielded several candidate molecular interactions. For example, we would test whether the Tfh canonical cytokine, IL-21, is required by using intravenous injection of blocking antibodies.

3. How do CD4+ CAR-Tfh or CD4+ CAR-Tctx engage the host immune system? To answer this question, we will adapt our model by using humanised NSG mice that have been prepared by prior infusion of human cord blood-derived CD34+ cells; these mice contain myeloid cells, NK cells, T and B cells allowing host immune-CAR-T interactions to be characterised(8). To evaluate how CD4+ CAR-Tfh or CD4+ CAR-Tctx engage the host immune system following infusion, we will use multi-parametric flow cytometry to define specific subsets (e.g., host B cells, T cells, macrophages, DCs, NK cells) at timed intervals in the spleen, lymph node and bone marrow. These findings will be compared to mice receiving CD8+ CAR-T or mock transduced CD4+ T cells. Deeper targeted interrogation by flow cytometry (e.g., for cytokines) and single cell RNA sequencing will further evaluate of the consequence of host-immune-CD4+ CAR T cell interactions. Where a candidate CD4-host interaction is identified, additional experiments will be directed at understanding how that interaction contributes to anti-tumour immunity (e.g., through in vivo cell depletion).

4. What are the fates of CD4+ Tfh-CAR or CD4+ Tctx-CAR following transfer? Using the humanised NSG model, we will examine the persistence, location, phenotypic stability and long-term function of adoptively transferred CD4+ CAR-Tfh versus CD4+ CAR-Tctx-cells. Survival of CAR-T will be evaluated by determining numbers CD4+ CD34-mimotope+ cells in the blood through serial sampling, and at timed intervals in the spleen, lymph node and bone marrow. Stability of the infused versus current phenotype (e.g., by measuring CXCR5, PD-1, ICOS for Tfh-like cells or SLAMF7, CTRAM, Granzyme B for T-ctx), as well as other markers of activation or memory differentiation will be tested. These studies could be combined with immunofluorescent confocal imaging to define where the surviving CAR-T are located. Where the two CD4+ CAR-T subsets diverge in relation to the above readouts, we will seek to define potential mechanisms that underpin these differences. For example, persistence of a CAR-T subset, but lack of phenotypic stability could relate to precursor-like properties of the infused subset. Lack of persistence of an individual subset could also relate to loss of critical niches (for example, through loss of B cells following anti-CD19 CAR-T) and this could be evaluated by using CD4+ CAR-T directed at alternative non-B cell targets.

Translational potential of the project

This project aims to identify the optimal CD4+ T cell states that can be incorporated into manufacture of CAR-T and design of other antigen-specific T cell therapies.

Training opportunities

The DPhil student will be trained in: (1) fundamental methods of immunology, specifically cell culture and manipulation, flow cytometry and single cell RNA sequencing (2) molecular biology, including methods for both gene editing/transfer (3) in vivo model design (4) computational biology and statistics. The training will be focussed on specific skill sets that are critical for developing immune therapies.

Ideal student background: The student will have a First Class or Upper Second Bachelor's degree (BSc or BA) in basic sciences or intercalated degree as part of a medical degree that will include exposure to immunology. Further laboratory experience is not essential but highly desirable. A prior laboratory or clinical publication record is desirable.

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5. Mechanisms and consequences of HLA-II expression in colorectal and endometrial cancer – David Church

Primary Supervisor: David Church
Additional Supervisors: Luciana Gneo
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract of the project
Presentation of antigen to T cells by HLA/MHC molecules is critical for tumour suppression and immunotherapy response. MHC-I molecules present antigen to $CD8^+$ cytotoxic T cells, are expressed by all nucleate cells and frequently downregulated in cancers as a mechanism of immune evasion. MHC-II molecules present antigen to $CD4^+$ cells and are normally only expressed by professional antigen-presenting cells. However, MHC-II expression is inducible in epithelial cells, with our recent analysis indicating that this occurs in 20-50% of colorectal and endometrial cancers. Epithelial MHC-II has been shown to present antigen to T cells in inflammatory bowel disease, but its function in cancer is little understood, and the consequences of its dysregulation in cancer undefined.

Research objectives and proposed outcomes
The objectives of this project are:
1. To define the frequency and consequences of MHC-II expression in colorectal and endometrial cancers and pre-cancers
2. To assist with the functional characterisation of MHC-II dysregulation in model systems

Research plan
The project aligns with a recent Cancer Research UK Senior Cancer Research Fellowship (PI: Church), and will benefit from the samples and data currently under generation in the Oxford LynchVax (PI: Church) and GO-PRECISE CRC-VAX (co-PI: Church) projects.

Correlates and candidate mechanisms
In collaboration with the Koelzer group (University of Basel), we have recently used AI-based image analysis to analyse MHC-II in nearly 3,500 colorectal and 1,500 endometrial cancers and a small number of LynchVax colonic precancers. Preliminary analysis reveals epithelial MHC-II expression coincides with the onset of dysplasia and is found in 20–50% of cancers; current work will define the clinical and molecular correlates of such expression along with its prognostic value. The student will have the opportunity to contribute to these analyses, and will lead the detailed study of MHC-II expression and its correlates in the large cohort of colonic precancers we are generating under the LynchVax and GO-PRECISE CRC-Vax industry collaborations. This work will leverage the exceptional multimodal data we will have for these samples including whole genome and transcriptome sequencing, immunopeptidomics and state-of-the-art spatial analyses of transcripts (Xenium 5k) and protein (phenocycler). This work will benefit from the multidisciplinary collaboration across the LynchVax and CRC-Vax groups.

Selected outputs from this work will include:

- Timing and frequency of MHC-II expression in colon and endometrial cancer
- Discovery and validation of genomic and transcriptomic features associated with MHC-II dysregulation (IFNg pathway activation, alterations in MHC-II pathway etc)
- Relationship between MHC-II expression and immune infiltrate, focusing on localisation and activation/exhaustion status of CD4, CD8 and other key cell types

Parallel work will investigate the functional role of MHC-II in cancer. This work aligns with the aim of the funded SCaRF (Church lab), and will benefit from the model systems to be generated under this work. As part of this work, the student will assist with the generation and characterisation of mice carrying conditional alleles of the MHC-II master regulator CIITA together in combination with other alleles we have shown induce MHC-II expressing tumours (e.g. Pole). Deep phenotyping will include analysis of precancers and cancers to define the consequences of MHC-II dysregulation on tumour development, immune infiltrate and depending on results, immunopeptidomics on primary tumours or tumour-derived organoids. Complementary human analyses will include generation of ex-vivo organoids, induction of MHC-II by eg IFNg, and detailed phenotyping including immunopeptidomics and potentially, co-culture with HLA-matched CD8 and CD4 T cells.

Translational potential of the project
The widespread use of ICB for colorectal and endometrial cancers and the 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 potential
The student will join a well-supported and highly collaborative research program, and benefit from teaching on all skills required. Bioinformatic analyses will be done under the supervision of the Church group, LynchVax and CRC-Vax team, and experimental work supported by dedicated postdoctoral scientist and research assistants in the Church laboratory.

6. Decoding Chromosomal Translocations in B Cell Malignancies: From Detection to Functional Interrogation – James Davies

Primary Supervisor: James Davies
Additional Supervisors: Sarah Gooding and I-Jun Lau
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract of the project
Chromosomal translocations—where DNA from different chromosomes is abnormally joined—are a hallmark of many blood cancers, particularly multiple myeloma and B cell malignancies[1] These structural changes often lead to activation of cancer-driving genes by placing them next to powerful regulatory elements, yet how these rearrangements occur and how they reprogram gene regulation remains poorly understood[2,3] This project aims to address three major challenges in this area: (1) improving the detection of chromosomal translocations using new long-read sequencing approaches, (2) understanding how these rearrangements affect gene cellular behaviour, and (3) developing genetic engineering tools to model translocations and study their effects in living cells and animal models. The insights gained will deepen our understanding of the biological role of chromosomal translocations as founder events in generating cancers, help improve diagnostics and potentially identify new treatment strategies for patients with blood cancers.

Research objectives and proposed outcomes
Objective 1: Develop a long-read, targeted sequencing method for detecting chromosomal translocations.
The student will use an established targeted long-read sequencing platform using PacBio technologies, to sensitively identify chromosomal translocations in multiple myeloma and B cell malignancies.[4] This approach aims to improve the resolution and accuracy of structural variant detection compared to current short-read methods or cytogenetics.[5] The focus will be to apply this approach to patient-derived tumour samples for precise characterisation of translocation breakpoints at nucleotide resolution. By mapping breakpoints across a cohort of samples provided by the MOSAIC study due to open later in 2025, as well as the OTMCbio Myeloma Biobank, the project will seek to identify genomic "hotspots" that are recurrently involved in translocations. These may coincide with regions of chromatin fragility, transcriptional activity, or underlying genetic variation such as single nucleotide variants (SNVs), which could predispose specific loci to breakage and rearrangement[6]. Together, these findings will improve understanding of the mechanisms driving translocation formation and inform future strategies for early detection and risk assessment.

Objective 2: Investigate how translocations affect gene regulation.
This project will explore how chromosomal translocations rewire gene expression and disrupt normal regulatory architecture. The student will use transcriptomic and epigenomic profiling techniques—such as RNA-seq, ATAC-seq, and chromatin conformation assays (e.g., Micro-Capture-C[7])—to explore how chromosomal translocations alter gene expression, enhancer hijacking, and 3D genome organisation in human cell lines and patient samples. These data will help to define how rearrangements rewire gene regulatory networks and contribute to malignant transformation.

Objective 3: Develop CRISPR-based systems to model translocations and study their impact.
This project will harness CRISPR-Cas9 genome engineering to recreate recurrent oncogenic chromosomal translocations seen in B cell malignancies —such as t(11;14) and t(4;14)—in human haematopoietic cells, enabling functional dissection of their oncogenic consequences. The student will design and optimise CRISPR tools to induce specific rearrangements at endogenous loci, facilitating accurate modelling of translocation events. The student will also develop in vivo mouse models by engrafting genetically modified haematopoietic cells into mice. These models will allow the induction of translocations in a developmentally controlled manner, specifically in B-cells at defined stages of differentiation, to explore how the cell of origin shapes disease

initiation, latency, and clinical phenotype. This approach will provide a powerful system for studying the early biological effects of translocations and for identifying novel vulnerabilities that may be exploited therapeutically.

Translational potential of the project

Chromosomal translocations are already used as diagnostic and prognostic markers in myeloma but current technologies can miss clinically important events[5,8,9]. By developing more sensitive detection tools and uncovering how these changes rewire the genome, this project could improve early diagnosis and enable more precise patient stratification. Furthermore, new models of translocation-driven cancers will provide valuable systems to test targeted therapies, including those exploiting gene regulatory changes or synthetic vulnerabilities. This work could inform future clinical strategies aimed at personalising treatment for patients with B cell malignancies.

Training opportunities

This project offers comprehensive training in cutting-edge techniques spanning molecular biology, genome engineering, and translational cancer research. The student will gain expertise in long-read sequencing technologies and the development of targeted capture approaches for breakpoint detection in patient-derived samples. They will be trained in high-resolution chromatin conformation assays, particularly Micro Capture-C, to study gene regulation around translocation breakpoints. Additionally, the project will provide hands-on experience in CRISPR-Cas9-based genome editing. Crucially, the student will also develop and work with in vivo models to investigate the role of translocations in different cell types and developmental stages. The student will also receive training in bioinformatic analysis of high thoughput sequencing data including ATAC-seq and chromosome conformation capture methods. The student will benefit from interdisciplinary mentorship across molecular biology, bioinformatics, and clinical research, with access to state-of-the-art core facilities and clinical sample resources at the MRC Weatherall Institute of Molecular Medicine and the Oxford Translational Myeloma Centre.

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7. A statistical analysis of nanoparticle captured p53 antibodies – Jason Davis

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

Abstract of the 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.

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 of the project
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.

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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8. Generation of selective immune checkpoint inhibitors with high on-target efficacy and low off-target toxicity – Omer Dushek

Primary Supervisor: Omer Dushek
Additional Supervisors: David Church
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract of the project
Immune checkpoint inhibitors (ICI) are soluble biologics that have been successful at treating a variety of cancer indications. However, these therapies also induce severe autoimmune toxicities in patients, and in some cases these are fatal. Indeed, the overall efficacy of the therapy is often associated with its overall toxicity. This trade-off between efficacy and toxicity has dramatically hampered the ability to increase the efficacy of ICIs. Here, we propose to explioit basic mechanisms of how T cells discriminate between non-self (e.g. pathogen and cancer antigens) and self antigens to develop Selective ICI (SICI) therapeutics that breaks this trade-off by enabling highly efficacious yet non-toxic immune response.

Research Objectives and proposed outcomes
T cells are activated to respond when their T cell antigen receptors (TCRs) recognise peptide antigens displayed on major histocompatibility complexes (pMHCs). They are not usually activated by normal cells that only present self-peptides because their TCRs have low-affinity to self pMHCs and because of co-inhibitory receptors such PD-1, which act as immune checkpoints preventing inappropriate activation.

T cells can activate to abnormal infected or cancerous cells because these cells present higher-affinity non-self pMHCs (e.g. viral or mutated peptides). However, cancers evolve to engage co-inhibitory receptors and hence inhibit T cell responses.

ICIs that block immune checkpoint receptors are widely used to treat many forms of cancer. However, while ICIs increase T cell responses to higher-affinity cancer antigens, they also increase T cell responses to lower-affinity self-antigens leading to autoimmune toxicity. This toxicity is often but not exclusively presented in the gastro-intestinal track.

Through our basic research on the mechanism of TCR discrimination, we have identified T cel surface proteins that selectively modulate responses to lower-affinity but not higher-affinity pMHCs. We will exploit these insights to develop selective ICIs that increase T cell responses to higher-affinity tumour antigens but not low affinity self-antigens, thereby improving the therapeutic index of ICIs.

The DPhil projects will facilitate collaboration between the laboratories of Omer Dushek (Dunn School), Ricardo Fernandes (COI at NDM), and David Church (CHG at NDM). Prof. Fernandes has expertise in the generation of bi-specific soluble reagents that can modulate the activity of surface proteins, Prof. Dushek has expertise in primary T cell assays for on-target efficacy and off-target toxicity including mechanisms that manipulate discrimination, and Prof. Church is a clinician scientist with expertise in the genetic underpinnings of antigen display, including on-target antigens in the gastro-intestinal track.

Translational Potential of the Project
The project aims to take the first step along the translational path by exploiting our basic understanding of self vs non-self antigen discrimination by T cells to reduce (or abolish) autoimmune toxicities in T cell-based therapies. These toxicities are often but not exclusively displayed in the gastro-intestinal track. By producing novel therapeutics that selectively reduce T cell resposnes to self antigens, the project will demonstrate the ability to produce highly effective but non-toxic therapeutics.

Training opportunities
The specific training will be catered to the experience and interest of the student. The project will involve theoretical training in the quantitaitive process of T cell antigen recognition, including the mechanisms of antigen discrimination, using mathematical modelling (no prior experience required). On the experimental side, the project can involve the molecular design of selective immune checkpoint inhibitors and their production and purification. The project will also involve isolation, expansion, and culture of primary human T cells along with T cell activation assays through co-culture where activation is assessed by flow cytometry, ELISAs, and imaging-based IncuCyte instruments. Training will also be provided in quantitative data analysis.

Ideal student background: No specific background is required. The project will involve cellular biology/immunology work, including primary human cell culture, cellular activation assays, and quantitative data analysis. The project can also involve molecular design and biochemistry of protein production/purification. General background in biology, biochemistry, biomedical sciences, medical sciences, etc are most suitable.

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9. Tackling Cancers Defective of High-Fidelity DNA Repair Mechanisms – Fumiko Esashi

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

Abstract of the project
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.

Research objectives and proposed 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.

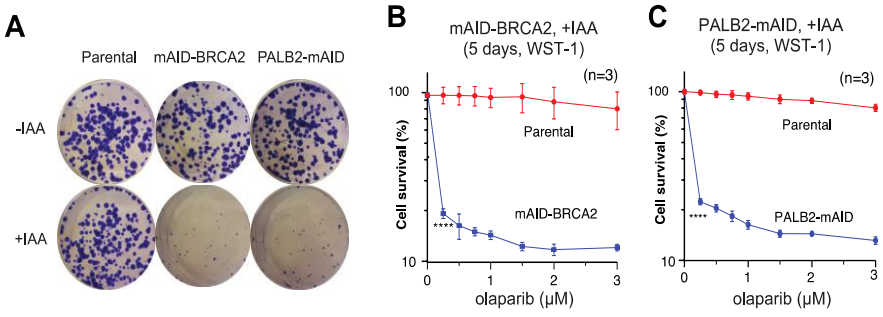

Figure 1. A. HCT116 mAID-BRCAs/PALB2-mAID cells were seeded in 6-well plates, and grown for 10 days with and without auxin (IAA). Colonies were then fixed and stained with crystal violet. **B-C:** HCT116 mAID-BRCA2/PALB2-mAID and parental HCT116 cells were first treated with IAA for 2 hours, and subsequently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

The genetic concept of 'synthetic lethality' or 'synthetic viability', involving the combination of mutations in multiple genes leading to cell death or growth, respectively, has gained rising attention in recent years for its potential for discovering new therapeutic targets in challenging cancers. Previous studies have relied on genome-wide loss-of-function screens in knockout cell lines. However, this approach has limitations, such as phenotypic changes obscured by secondary mutations. This project tackles these shortcomings by utilising the auxin-inducible degron (AID) technology (**3**) to conditionally deplete endogenous BRCA2 or PALB2 in MMR-defective HCT116 cell lines. This allows for highly-specific examination to uncover the direct impact of 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 characterise the cellular and molecular functions of these hits.

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

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

Translational potential of the project

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

Training opportunities

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

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

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10. Dissecting the relationship between cytomegalovirus and tumour immunosurveillance in the skin – Benjamin Fairfax

Primary Supervisor: Benjamin Fairfax
Additional Supervisors: Paul Klenerman
Eligibility: Track 2 applicants are eligible to apply for this project

Abstract of the project
Cytomegalovirus (CMV) is a betaherpes virus with a seroprevalence of approximately 50% in the UK and >80% worldwide. CMV has profound effects on immunity, leading to T cell memory inflation, with altered T cell clonality and gene expression. We have recently shown that CMV infection is associated with multiple positive prognostic factors in metastatic melanoma, including reduced neutrophil:lymphocyte ratio and reduced numbers of circulating regulatory T cells. In addition, we find that CMV interacts with melanoma epidemiology in a manner in keeping with protection against metastatic *BRAF* mutated melanoma, whilst delaying presentation by up to 9 years of metastatic *BRAF* wild-type melanoma. The mechanism whereby this occurs is unclear, although we postulate CMV alters resident skin immune cell subset frequencies and function, with consequences in immuno-surveillance. This DPhil will explore the effects on the skin immune microenvironment in the context of CMV infection, as well as other organs. The relationship between T cell clonality, and tissue-specific immunosurveillance will also be examined using a mixture of bioinformatic and immunological techniques including spatial transcriptomics and functional immunology.

Research objectives and proposed outcomes
1 To characterise variation in skin immunity in patients with melanoma pre and post checkpoint immunotherapy
2 To explore interactions of these features with patient germline genetics and environmental factors including CMV infection

Translational potential of the project
CMV is the first environmental factor found to influence both response and toxicity to checkpoint immunotherapy. The mechanism whereby this occurs is yet to be deduced, but may provide insights into advanced therapeutics and inform treatment stratification. The data analysed are all within the OxCITE project (https://www.cancer.ox.ac.uk/research/projects/oxcite) and therefore of high translational value.

Training opportunities
Successful candidates will gain expertise in single cell transcriptomics, population genetics, flow cytometry, spatial transcriptomics, eQTL amongst other techniques in use within the group. They will learn to collect and analyse data as well as present it in person to group meetings, and national/ international events with a view to publishing work in high impact journals.

Ideal student background: The successful candidate will be required to be enthusiastic, reflective, highly-organised, tenacious and patient. The position is suitable for undergraduates who are taking time out from a medical degree to undertake a DPhil – as per the supervisor – as well as both equally for clinically qualified and non-clinical trainees. The group-leader is happy to discuss the project informally with any potential applicants.

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11. Agonised Peptide Identification in Cancer Specific T Cell Immunity – Ricardo Fernandes

Primary Supervisor: Ricardo Fernandes
Additional Supervisors: Tao Dong
Eligibility: Track 2 applicants are eligible to apply for this project

Abstract of the project
T cells are central to immune surveillance, using their T-cell receptors (TCRs) to scan peptide–MHC complexes and mount targeted responses against infected or malignant cells. The quality and magnitude of these responses are shaped by signals from both the TCR and co-receptors. Advances in single-cell sequencing now enable the identification of TCRs from clonally expanded, tumour-infiltrating lymphocytes (TILs), yet defining their antigen specificity remains a major bottleneck, particularly due to the inherent cross-reactivity of TCRs, which can recognise multiple distinct peptides. This limits our ability to rationally exploit TCRs in immunotherapy. This project aims to develop a robust framework to identify tumour-related agonist peptides recognised by TIL-derived TCRs from solid tumours. To achieve this, we will pursue complementary strategies to build a high-resolution connectivity map linking TCR sequences to their cognate tumour-associated peptide antigens.

Research objectives and proposed outcomes
Identifying the peptide antigens recognised by T-cell receptors (TCRs) remains a major challenge due to the extraordinary diversity and complexity of the three molecular components involved: peptide ligands, the TCR repertoire, and the major histocompatibility complex (MHC). This project aims to define the antigenic landscape recognised by TCRs derived from clonally expanded and tumour-reactive T cells and uncover the principles linking TCR sequence, structure, and function. The analysis will focus on T cells isolated from melanoma, lung and breast cancer. Specifically, we aim to: (1) deorphanise TCRs from tumour-infiltrating lymphocytes using affinity and functionalscreening platforms; (2) explore antigen reactivity across related TCRs in public databases, supported by structural studies; and (3) engineer high-potency TCRs with refined specificity (see **Fig. 1**). These efforts will yield a comprehensive dataset linking TCR sequences to tumour-associated peptides, structural features, and functional responses, with direct applications in immunotherapy design and a deeper understanding of T cell-mediated tumour recognition.

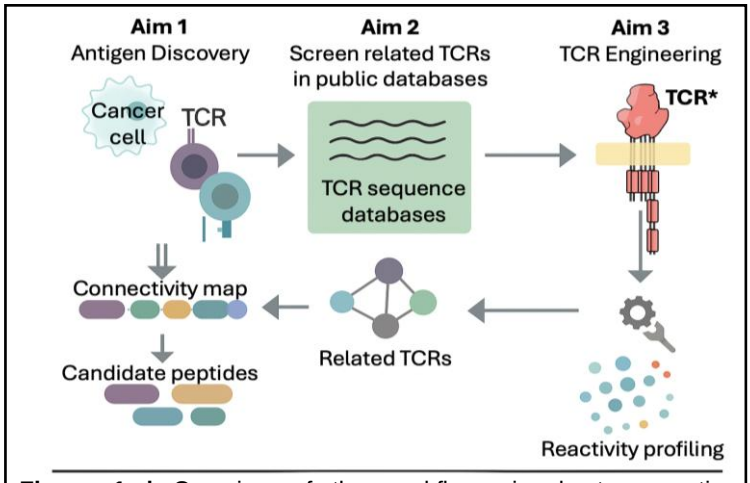

Figure 1 | Overview of the workflow aimed at generating comprehensive datasets linking TCR sequences to tumour-associated peptides, structural features, and functional responses

Aim 1: Deorphanising tumour-reactive TCRs through affinity and functional screening
Our first goal is to identify cognate peptide ligands, including mimotopes and tumour-associated peptides for clonally expanded tumour-infiltrating lymphocytes (TILs). We will employ two complementary platforms: affinity-based selection using peptide-MHC yeast display libraries, and a functional screen using engineered T cells carrying sensitive reporter systems. The combination of affinity- and activity-based selection strategies allows us to capture both high-affinity binders and physiologically relevant functional agonists. Using custom-

built computational algorithms, we will map identified mimotopes to candidate wild-type peptides from the human proteome, iteratively refining these predictions through cycles of experimental validation.

Aim 2: Exploring TCR-antigen connectivity across public repertoires
To extend our findings beyond individual TCR sequences, we will mine large-scale public TCR sequence databases (e.g. the Observed TCR sequence space) to identify clonotypes that are closely related to the tumour-reactive TCRs identified in Aim 1. These related sequences will be expressed and evaluated using the same affinity and functional screening pipelines. Our objective is to uncover patterns of antigen reactivity across similar TCRs, and to identify sequence features that predict tumour specificity. This analysis will be complemented by structural studies of selected TCR-pMHC complexes to mechanistically link sequence variation to binding geometry and functional output.

Aim 3: Engineering high-potency TCRs with reduced cross-reactivity
Building on insights from Aims 1 and 2, we will select a subset of high-value TCRs for structure-guided engineering. Using site-directed mutagenesis, we will introduce targeted amino acid substitutions and assess their impact on antigen recognition, potency, and specificity. These experiments will help clarify how subtle sequence changes influence TCR behaviour, providing a fundamental understanding of the sequence–function relationship. In parallel, this work will enable the generation of enhanced TCRs with improved therapeutic potential and reduced risk of off-target effects.

In parallel with our antigen discovery and TCR mapping efforts, we will perform single-cell transcriptomic analysis of relevant T cell populations expressing TCRs under investigation. This will add a critical phenotypic dimension to our dataset, enabling us to link TCR sequence and antigen specificity to functional state, activation status, and differentiation trajectory. By integrating agonist peptide identification with transcriptional profiling at single-cell resolution, we aim to dissect the roles of diverse T cell subsets during anti-tumour immune responses.

Together, these three aims will produce an integrated map linking TCR sequence, structure, reactivity, and function. This work will not only deepen our understanding of T cell recognition in cancer, but also inform the design of safer and more effective TCR-based therapies. Importantly, the methods, datasets, and engineered TCRs generated will be made available to the broader scientific community, with potential applications extending beyond oncology to infectious diseases and autoimmune disorders.

Translational potential of the project
This research programme is designed to bridge fundamental T cell biology with translational immuno-oncology. By identifying tumour-reactive TCRs and mapping their interactions with cancer-related agonist peptides, we aim to both elucidate mechanistic principles of antigen recognition and open new avenues for therapeutic development. The datasets generated through this work, including TCR-peptide connectivity maps, functional and structural profiling, will provide a valuable resource for guiding the selection of optimal TCRs for adoptive cell therapies or for further engineering as affinity-enhanced TCRs. Furthermore, the identification of agonist peptides may lead to the discovery of previously uncharacterised antigens associated with tumour growth, offering opportunities to expand the known tumour antigen landscape. Together, these advances position the project to contribute directly to the design of next-generation immunotherapies and improve our capacity to target cancer with precision.

Training opportunities

The student will gain hands-on expertise in a range of cutting-edge techniques, including yeast and mammalian display technologies, peptide–MHC library generation, TCR engineering, high-dimensional flow cytometry and T cell phenotyping and manipulation. The project also offers the opportunity to develop skills in molecular modelling and structural interpretation of TCR–pMHC interactions. Exposure to translational pipelines, including TCR validation, antigen screening, and therapeutic candidate selection, will provide valuable insight into the clinical development process for immunotherapies. The student will benefit from mentorship within a collaborative, cross-disciplinary environment that includes close interactions with the labs of Professors Tao Dong, Omer Dushek and Charlotte Deane. Attendance at departmental seminars, national and international conferences, and specialist training workshops will further support the student's scientific and professional development, preparing them for diverse future careers in academic research, biotechnology, or clinical translation.

Ideal student background: The candidate will be highly motivated, with a strong interest in immunology and the role of T cells in recognising and eliminating cancer cells. Prior hands-on experience in molecular cloning, cell culture, and/or protein expression is advantageous but not essential. A background in immunology, molecular biology, or related fields is preferred. The project requires a willingness to engage with both experimental and computational aspects of T cell research. Full training will be provided for all laboratory and analytical techniques required, making this a valuable opportunity for skill development in translational immunology.

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12. Integrating spatial transcriptomics, molecular epidemiology and digital pathology to study Renal Cell Carcinoma – Kezia Gaitskell

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

Abstract of the project
Kidney cancer is the sixth most common cancer in the UK with a steady increase in incidence over the last decade [1]. 80-85% of kidney cancers are renal cell carcinomas (RCC), which encompass many different histological subtypes [2]. Immunological and genomic techniques have demonstrated the significant inter- and intra-tumoural heterogeneity in the tumour immune, angiogenic and metabolic landscape [3], which is reflected by the heterogeneity in treatment response across different subtypes of RCC [4]. Tumour heterogeneity poses significant challenges to diagnosis and biomarker development for risk stratification, predicting therapeutic response, and prognostication. Discovering heritable genetic variants of RCC has advanced our understanding of the biology of RCC and informs clinical genetic testing; however, it is highly likely that a significant part of this heritability, particularly of rare variants, is undiscovered [5].

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 and linkage to health records [6]. 5000 cases of kidney cancer have accrued after 20 years of follow-up in the cohort. Scanned digital pathology slides, archival tumour tissue, and blood samples with derived exome sequencing data, are available for a subset of patients; genetic data from blood samples are also available from several other large cohorts. These will allow for molecular and digital pathology, and molecular epidemiology analyses, respectively.

We aim to combine large-scale epidemiological data on several large cohort studies, with rich molecular and digital pathology data on a subset of cases, to investigate the biological mechanisms underlying inter- and intra-tumoural heterogeneity, discover the missing heritability of RCC particularly of rarer subtypes, and improve diagnostic and prognostic accuracy. This approach will provide an unparalleled resource with important clinical implications for prevention, genetic testing, prognostication and treatment options.

Research objectives and proposed outcomes
Evaluate the utility of spatial transcriptomic (ST) data generated from archival renal cell carcinoma (RCC) tissue from a subset of MWS participants.
Previous pilot work has used archival samples of RCC to generate digital pathology slides and tumour tissue micro-arrays from a subset of MWS participants with kidney cancer (~n=50), which have been characterised using immunohistochemistry and spatial transcriptomics. Since nucleic acid quality is affected by environmental and (possibly) patient factors, we will first assess the utility and quality of ST data in relation to storage duration and patient characteristics, to inform future archival tissue-based epidemiological research from this and similar cohorts. Subsequent analyses will include spatial visualisation of gene expression, deciphering intercellular interaction and mapping spatial transcriptomic variations to different tissue morphology using data generated from different subtypes of RCC. These will provide important novel data on detailed molecular profiles of different subtypes of RCC, including information on rarer subtypes and tumour heterogeneity, from a large representative cohort of women aged 50 and over. Opportunities exist to expand this work with additional samples.

Identify new germline variants associated with the risk of RCC using exome sequencing data from blood samples from several large prospective cohorts.

Whole exome sequencing (WES) data from blood samples are available in a subset of MWS participants (n ≈ 50,000, of which ~230 are RCC cases). We will collaborate with Dr Karl Smith-Bryne, a molecular epidemiologist, and use WES data to investigate the association between the risks of RCC and rare germline variation through gene burden testing. We will also perform meta-analysis of WES data from the MWS, the UK Biobank and the Mexico City Prospective Study to detect rare germline variants and to evaluate population-level variation in the risks of RCC. These variants will be taken forward for functional characterisation, such as by spatial transcriptomics, on a tissue level to explore whether the identified germline variants correspond to expression differences in tumour tissue. Additionally, polygenic risk scores (PRS), will be derived to assess the impact of common variation and test whether high common burden associates with spatial transcriptomics features. This will provide information on the heritability of RCC and the biological significance of common and rare germline variants, which carries significant implications for genetic testing in clinical practice.

Validate existing machine-learning based algorithms for diagnosis and classification of RCC and generate a novel algorithm using data from our cohort.
We aim to perform detailed digital pathology analyses, based on morphological features and molecular data, using AI/machine-learning approaches on digital whole-slide pathology images. This will be undertaken in collaboration with Professor Jens Rittscher's group at the Big Data Institute. This will enable independent validation of published machine-learning based algorithms for RCC classification using a large population-based cohort in the UK. We will also explore image-based correlates of immunophenotypic profile and the newly identified molecular changes of different subtypes of RCC. We aim to combine digital pathology with histological, immunophenotypic and molecular data to generate a multifaceted algorithm to advance the diagnostic and prognostic accuracy of RCC. This project will foster important collaborations between pathologists, epidemiologists, data scientists and engineers within and between centres, by utilising data from large population-based studies.

Translational potential of the project
This project has a high potential for clinical translation and enhancing patient care. Identification of susceptible germline variants and high-resolution spatial molecular characterisation will improve our understanding of tumour biology and inform genetic prediction of kidney cancers. The use of machine-learning approaches to correlate tumour morphology with molecular pathology markers will improve diagnostic and prognostic accuracy, which will pave the way for personalised treatment options.

Training opportunities
The student will join a diverse team of pathologists, epidemiologists, data scientists and engineers, receiving comprehensive training in basic and molecular epidemiology, statistical analysis of complex linked health records data, analysis of transcriptomic data, molecular and digital pathology, and machine-learning methods as applied to digital pathology. There will also be opportunities for developing communications and leadership skills through presenting at international conferences, writing manuscripts for publications, and contributing to public engagement activities.

Ideal student background: The selected individual for this project should either have strong quantitative skills, preferably including familiarity with either epidemiology and statistics and/or machine-learning methods, or substantial experience in histopathology (ideally both), in addition to a basic understanding of genetics. Experience of digital pathology would be advantageous but not essential. Strong communication and collaboration skills are essential to facilitate multidisciplinary team working.

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13. Deciphering the phagocytic and antigen presenting landscape within Hepatocellular Carcinoma – Felipe Galvez-Cancino

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

Abstract of the project

Hepatocellular carcinoma (HCC) is the fourth most common cancer worldwide and one of the fastest growing in the UK. The HCC leukocyte tumour microenvironment (TME) is rich in phagocytes, including Tissue-Resident Kupffer cells (TRMs), monocyte-derived macrophages (MDMs), monocytes, neutrophils, and dendritic cells (DCs). Whether we can engage these phagocytes to kill tumour cells or other components of the tumour microenvironment and thus control or even clear HCC needs to be addressed. While antibody-dependent cellular cytotoxicity (ADCC) is usually considered the primary mechanism underlying the activity of depleting antibodies (mAbs) targeting antigens on the surface of tumour cells, these also trigger antibody-dependent cellular phagocytosis (ADCP) that contributes to their activity. Harnessing ADCP enhances the activity of rituximab, a depleting mAb targeting B-cell lymphomas, while enhancing T-cell priming.

However, the cells that drive ADCP, the mechanisms that restrict this process within solid tumours, and whether the antigens derived from the phagocytosed cells are cross-presented, priming novel clones of $CD8^+$ T cells are not fully understood. The work of our team has recently focused on understanding the phagocytic populations within solid tumour and the role of cross-presenting

identification of $FcgR^{hi}$ monocyte-derived macrophages ($FcgR^{hi}$ MDMs) as key mediated of ADCP and the study of cross-presentation as a fundamental mechanism to elicit $CD8^+$ T cell priming within tumours. A better understanding of ADCP and cross-presentation within the TME has the potential for devising novel depleting antibody combinations that unleashes the potential of the myeloid compartment in HCC to clear tumours. This proposal addresses three critical questions:

1. What is the phenotype, dynamics and spatial distribution of FcγR-expressing cells in HCC?
2. Which cells mediate ADCP, and whether they cross-present antigens from the phagocytosed tumour cells?
3. How can ADCP be best exploited therapeutically in HCC?

Hypotheses.
1. $FcγR^{hi}$ MDMs are highly phagocytic and essential for the activity of depleting antibodies.
2. Enhancing the phagocytic capacity of $FcγR^{hi}$ MDMs will improve the efficacy of depleting antibodies while cross-priming phagocytosed antigens and eliciting novel clones of tumour-reactive $CD8^+$ T cells.

Aims. (1) Assess the FcγR landscape in HCC in mice and humans and characterise the dynamics, phenotype and transcriptome of FcγR-expressing leukocytes; (2) Interrogate which cells mediate ADCP in vivo and whether this mechanism leads to antigen cross-presentation; and (3) evaluate novel immunotherapy agents and rational combinations to treat HCC.

Methods. Mouse models of multifocal HCC and human surgical HCC samples. Single-cell technologies will reveal transcriptional profiles and phagocytic receptors in human and murine myeloid cells of HCC. Novel multispecific mAbs targeting tumour cells, alongside combinatorial approaches, will be evaluated using humanised mouse models and human tissue samples.

Research objectives and proposed outcomes

What is the phagocytic capacity and origin of FcγR^{hi} MDMs?
Depleting anti-Glypican-3 antibodies will be tested using an HCC mouse model expressing Glypican-3. According to Hypothesis 1, FcγRhi MDMs exhibit superior phagocytic activity. To quantify the phagocytosis of tumour cells, you will use TdTomato expressing tumour cells in our mouse model of HCC, as this fluorescent protein allows the detection of tumour cells engulfed by phagocytes. If ADCP is a property exclusive to MDMs, therapeutic strategies must enhance MDM activity while considering their functional differences from tissue-resident macrophages.
The impact of anti-Glypican-3 will be further assessed using single-cell and spatial transcriptomic analyses. These will: 1. inform the enrichment pf phagocytic and antigen cross-presentation signatures within FcγR^{hi} MDMs as a measure of their phagocytic and cross-presenting potential. 2. Determine the FcγR^{hi} MDMs tumour niches and identify cellular interactions (e.g., Treg-MDM). The cellular origins and dynamics of FcγRhi MDMs will be analysed using Photoconvertible Kaede mice to distinguish resident macrophages (TRMs) from newly infiltrating myeloid cells (MDMs, dendritic cells, and neutrophils) within the tumour. This analysis will be complemented with monocyte fate-mapping (Ms4a3-RFP), allowing tracking of monocyte-derived cells and their differentiation into FcγR^{hi} MDMs[18].

Do FcγR^{hi} MDMs mediate ADCP that results in antigen cross-presentation following anti-Glypican-3 treatment? Aim 1 will determine if FcγRhi MDMs are the most phagocytic. According to Hypothesis 1, macrophage depletion should abrogate the effect of anti-Glypican-3 antibodies. You will investigate the roles of key immune populations using depleting antibodies targeting neutrophils, NK cells, monocytes and Clodronate liposomes to eliminate macrophages. To differentiate between MDMs and TRMs, you will delete the FcRγ-chain (FcRγ-flox) on MDMs (Ms4a3-Cre) and TRMs (Clec4f-Cre). To verify that human (hIgG1) anti-Glypican-3 antibodies interact effectively with cells expressing human FcγRs and HCC, you will utilise humanised FcγR mice. Phagocytosis of Glypican-3^{+} tumour cells is expected to result in antigen cross-priming, activating new tumour-specific T-cell clones that will be assessed by TCR-sequencing. You will use peptide-restimulation and MHC-tetramers to assess the generation of tumour-reactive CD8+ T cells, including those that react to Glypican-3.

To validate the findings in human cells, you will purify FcγR-expressing cells from human HCC, including resection and ascites and perform in vitro ADCP assays using human anti-Glypican-3 (hIgG1) antibodies and Glypican-3+ tumour cells. Finally, you will use the liver perfusion system within the OrganOx device. to understand whether FcγR^{hi} MDMs undergo phenotypic changes by flow cytometry or spatial transcriptomics and mediate ADCP following the administration of human anti-Glypican-3 mAbs.

Enhancing the phagocytic and cross-presenting capacity of FcγR^{hi} MDMs. In addition to using exploring the effects of anti-Glypican-3 depleting antibodies you will design and test novel therapeutics with the potential of enhancing ADCP and antigen cross-presentation.

Translational potential of the project
Describe the relevance of the project to cancer research or patient care.
ADCP and antigen cross-priming are critical to the function of therapeutic antibodies in the treatment of cancer. This proposal will interrogate how these mechanisms are controlled in HCC. Using this knowledge, you will then develop and test new therapeutics harnessing ACDP with the potential to be translated into clinical trials for these patients.

Training opportunities
The student will perform mouse work using cutting-edge single cell and spatial transcriptomics techniques. Furthermore, with our clinical collaborators you will collect human tissues to assess the different FcgR populations within human tissues and their interactions. Finally, this program expects to generate rich datasets that will require the student to learn and develop bioinformatics expertise.

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14. Dissecting the microenvironment of liver and lung metastatic colorectal cancer using spatial transcriptomics and human tissue models – Alex Gordon- Weeks

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

Research Objectives and Outcomes.

Metastatic colorectal cancer (CRC) has a poor outcome with relatively limited therapeutic options. Surgery offers the best chance of long-term cure but is an option for few patients. Cancers in the liver are particularly insensitive to immunotherapeutics and the mechanisms behind this remain poorly understood. This project aims to uncover therapeutic vulnerabilities that would sensitise to immunotherapy through two **primary objectives**:

1. **Microenvironmental analysis of CRC metastases using single cell, spatial transcriptomics**.

2. **Mechanistic investigation of observations from (1) using human tissue slice models**.

Objective 1.

We have developed spatial single-cell transcriptomics capability allowing us to examine the tumour microenvironment at unprecedented level. We use this to investigate mechanisms of immune exclusion and exhaustion in metastatic CRC samples (manuscript under submission). **Figure 1** provides an example of where we define hepatocyte zonation, a conserved spatial tissue of the liver microenvironment using the technology. The candidate will use images from patients with metastatic CRC to the liver and lungs, comparing the different metastatic sites and analysing the spatial relationship between cell populations and their transcriptomes.

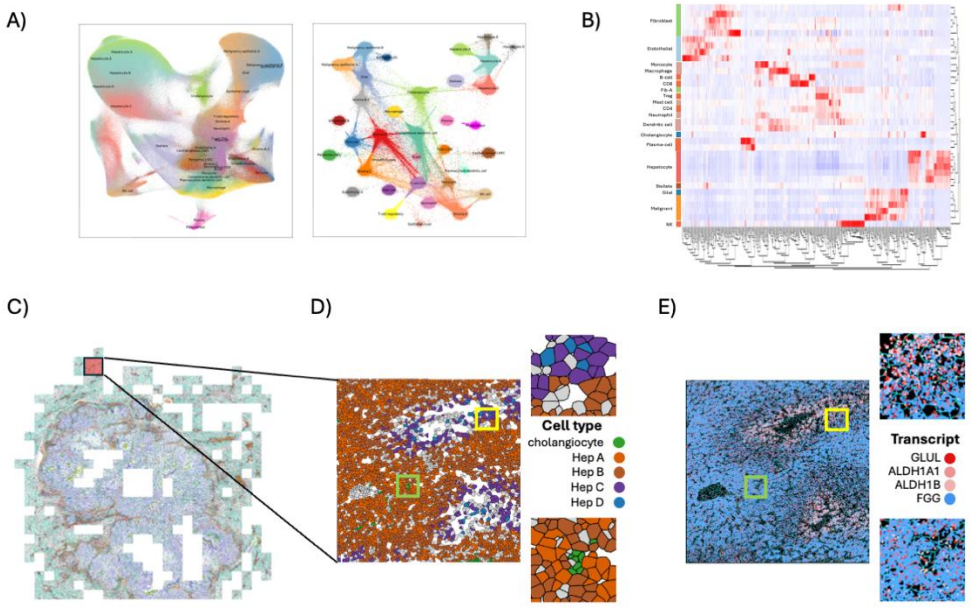

Figure 1. Single-cell spatial transcriptomics using the NanoString CosMx 6K gene panel enables delineation of hepatic zonation. A) UMAP (left) and flightpath (right) of cell clustering from 4 whole-slide CRLM tissues including the cancer and background liver. **B)** Heatmap of top 30 differentially expressed genes across each cell type. **C)** Spatial plot of every cell type colour coded across one of the whole slide images. **D)** Hepatocyte and cholangiocyte assignment based on transcriptional data demonstrates zonation with Hep A and B in the peri-portal zone adjacent to cholangiocytes (green) and Hep C and D in distant to the cholangiocytes in the peri-central zone. **E)** Zonation is confirmed through observation of transcripts specific for detoxification (GLUL, ALDH1A1 and ALDH1B) in the peri-central zone and clotting factor production (FGG) in the peri-portal zone.

Objective 2.

Spatial biology provides unparallelled observational data for hypothesis generation. To provide mechanistic insights, we will use tissue slice perfusion. Here, cancer samples resected from patients are sliced and cultured for up to 7 days in flow chambers providing multiple replicates of the same tumour on which therapeutics can be tested (**Figure 2**). Guided by observations in the spatial transcriptomics data, we will test combination therapeutics and assess their biological effects using multiplexed immunohistochemistry, flow cytometry and single cell sequencing.

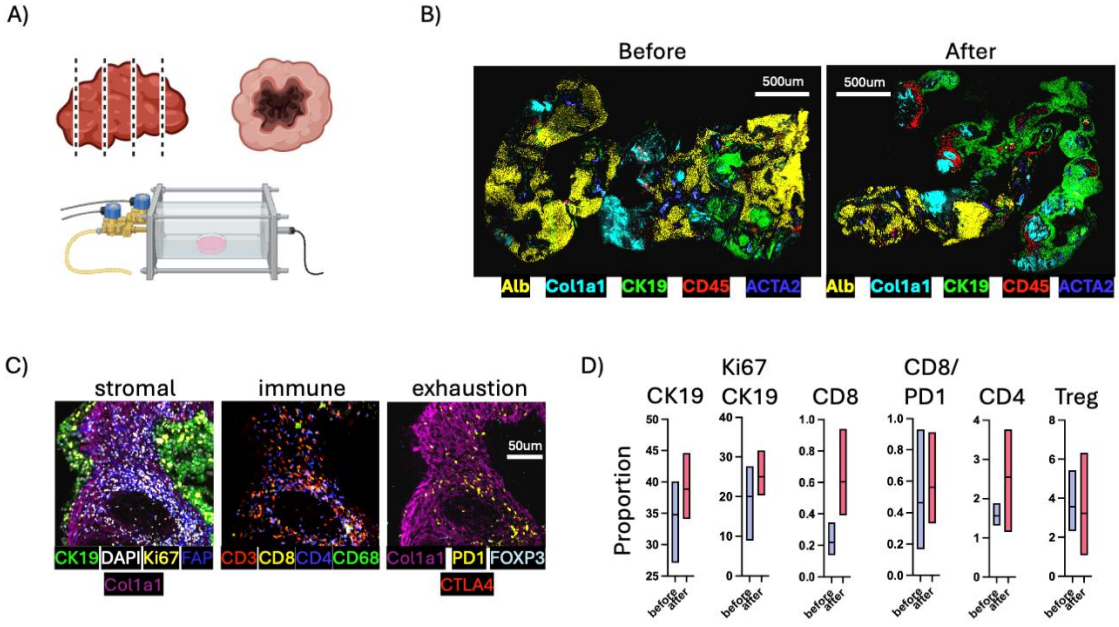

Figure 2. A human metastatic CRC slice model. A) Tumours are sliced after surgical excision and cultured in flow chambers (Bio-render). **B)** Multiplexed immunohistochemistry (cell-DIVE) of a slice before and after 7 days of culture. **C-D)** Broad maintenance of the stromal and immune tumour compartments as demonstrated through quantification of multiplexed images. Note ongoing T-cell exhaustion (PD1/CTLA4) and cancer cell proliferation (Ki67).

Translational Potential.

The technologies you will develop provide unparalleled ability to represent several features of human liver cancers not currently afforded by murine or cell culture systems. These include the full complement of tumour microenvironmental cell types and mapping of cellular responses to treatment over time. By focusing solely on human samples, the research addresses tumour heterogeneity in human cancer, inferring subtype-specific

therapeutic responses. These features will lead directly to a personalised approach to trial design and a de-risking of early-phase therapeutic studies which we are well placed to carry out subsequently in Oxford.

Training opportunities.

These are broad and can be tailored to the applicant's specific career aims. They range from bioinformatics approaches to wet-lab techniques as per objective 2. The spatial transcriptomics technique is a cutting-edge technology and the pipelines that we have developed for image analysis are continually developed and refined. Development of these skills will provide the applicant with a skill that will make them highly competitive in post-doctoral applications. The student will work with a spatial bioinformatician in the supervising laboratory for direct training. The applicant will be trained in the liver slice technique which is central to our lab's current workflow.

15. Investigating changes in RNA-binding proteins in hypoxia – Ester Hammond

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

Abstract of the project

Hypoxia (conditions of insufficient oxygen) is a common feature of the tumour microenvironment leading to therapy resistance and poor patient prognosis. Hypoxic cells undergo transcriptional stress, which we have recently shown to involve R-loop accumulation, nucleolar reorganisation and repression of rRNA synthesis. Furthermore, our previous study demonstrated that increasing R-loop accumulation in hypoxia led to significant changes in expression of RNA binding proteins. These data led us to hypothesis that the study of RNA-binding proteins in the context of hypoxia-induced transcriptional stress could identify both novel biology and therapeutic targets. Therefore, we set out to identify novel RNA-binding and/or R-loop-binding proteins that regulate the transcriptional stress response. We successfully isolated RNA-binding proteins from cells and generated samples for mass spectrometry to quantify changes in the RNA-bound proteome between normoxic and hypoxic cells. Importantly, hypoxic samples were also prepared with cells overexpressing RNase H1, an endonuclease that degrades the RNA strand of R-loops, to investigate RNA-bound proteome differences between hypoxic cells with and without significant R-loop levels. Approximately 70 proteins were identified which showed changes in RNA binding activity in hypoxic conditions, of these 20 also changed in an RNase H1 dependent manner suggesting a role at R-loops. The aim of this project is to further define the roles of specific RNA-binding and/or R-loop-binding proteins in hypoxia and identify potential targets for novel therapeutic strategies.

Research Objectives and proposed outcomes

Initial research objectives (likely the first 12-18 months) are detailed below, longer term objectives will be determined by the supervisory team and student in response to the data obtained.

1. Target selection

Proteins of interest will be determined based on the available literature, availability of reagents (in particular validated antibodies), the existence of small molecule inhibitors, dysregulated expression in human cancers and reported links to hypoxia biology. Outcome – a shortlist of 5-10 proteins of interest.

2. Validation oxygen-dependent RNA binding activity/R-loop association

Using Orthogonal organic phase separation (OOPS), we will verify that the RNA binding of the candidate proteins changes in an oxygen dependent manner. This will include the use of a range of physiologically relevant oxygen concentrations (0.1-2% O_2). Furthermore, we will look for evidence for an association with R-loops (stable hybrids formed between DNA and RNA) using the S9.6 antibody to immunoprecipitate R-loops and associated proteins. Outcome – validation of RNA binding proteins which are regulated in an oxygen dependent manner.

3. Impact on nuclear and cytoplasmic damage

siRNA mediated depletion of the target protein (or inhibition where an inhibitor is available) will be used to determine the impact of the protein on DNA damage in both normoxic and hypoxic conditions. For nuclear

damage we will use immunofluorescence to detect 53BP1 foci and, as a marker of possible cytoplasmic damage, qPCR for interferon stimulated genes (ISGs) and type I interferons. Outcome – identification of potential target(s) which could be exploited to increase therapy response in hypoxic tumours.

Translational potential of the project

The goal of this project is to identify novel biology which can be exploited to improve therapy response. Specifically, we aim to identify targets which when lost or inhibited lead to better outcomes for hypoxic tumours treated with standard therapies (e.g. radiotherapy). Prof Blagden's involvement as a collaborator is absolutely essential to this project as she brings significant expertise in targeting the RNA binding protein, LARP1 for therapy and will be able to assist with this aspect of the project.

Training opportunities

The student can expect training in molecular/cell biology, biochemistry and proteomics. In addition, preclinical testing of promising candidates and understanding of the challenges to successful clinical translation. The student is expected to spend time in both the Hammond and Mardakheh labs, the latter in particular for biochemistry/proteomics training.

Ideal student background: A background or clear interest in cancer biology.

References

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16. SHLD2 as a Determinant of Response to Polθ Inhibition Combined with Radioligand Therapy – Geoff Higgins

Primary Supervisor: Geoff Higgins
Additional Supervisors: Edward O'Neil
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract of the project
DNA polymerase theta (Polθ) is a DNA repair polymerase known for its role in micro-homology mediated end joining (MMEJ) and whose disruption is known to cause synthetic lethality in homologous recombination (HR) deficient tumours. We have previously shown that Polθ inhibition (Polθi) renders cells sensitive to radiotherapy (RT) treatment both *in vitro* and *in vivo* independently of the HR proficiency status[1]. We have recently conducted a CRISPR screen and established that loss of SHLD2 (a key component of the Shieldin complex which regulates DNA end resection) increases cancer cell sensitivity 1) to radiotherapy alone, and 2) to Polθi in combination with radiotherapy. Of note, SHLD2 is frequently deleted in prostate cancer.

We are now seeking to establish the mechanism by which SHLD2 exerts these effects. Since PSMA targeted radioligand therapy is now used in the treatment of metastatic prostate cancer, we will also investigate whether SHLD2 KO cells are more sensitive to radioligand therapy as a single-agent, and in combination with Polθ inhibitors.

Research objectives and proposed outcomes
Polθi are novel small-molecule drugs that target DNA damage repair (DDR) [1,2,3], and are currently being tested in clinical trials. We have previously shown that these agents cause tumour-selective radiosensitisation[1]. To identify putative biomarkers to predict response to Polθi plus radiotherapy, we undertook a CRISPR screen of DDR genes (Fig 1A) which found that loss of SHLD2 and other components of the Shieldin complex rendered DLD1 cells more sensitive to Polθi plus RT (Fig 1B).

Since SHLD2 function is disrupted in up to 10% of prostate cancers, we created SHLD2 KO cells in the DU145 and 22RV1 prostate cancer cell lines and showed that the Polθ inhibitor ART558 markedly increased their sensitivity to radiation, thereby confirming the CRISPR screen results (Fig 1C and D). As further validation, we demonstrated that restoration of SHLD2 function in these KO cell lines reversed the Polθi induced radiosensitisation (Fig 1E).

Subcutaneous xenograft tumours were established in NRG mice using both the DU145 WT and DU145 SHLD2 KO tumours. Although Polθi alone and combined with RT had little or no effect on the WT tumours (Fig 1F), the SHLD2 KO cells were significantly more sensitive to radiation alone with a marked synergistic effect in combination with Polθi (Fig 1G).

We are now seeking to understand the mechanism by which SHLD2 disruption renders tumour cells more sensitive to Polθi-mediated radiosensitisation. Key areas of interest we will explore include whether SHLD2 influences Polθ-mediated repair by modulating DNA end resection, the interplay between SHLD2 and Polθ in microhomology-mediated end joining (MMEJ), and the relationship between Polθ and SHLD2 in post-replicative gap filling and the response to replication stress.

We hypothesise that prostate cancer patients whose tumours show loss of SHLD2 function, will be more responsive to radiotherapy (including PSMA RLT), and that these tumours will be radiosensitised by Polθi. We will therefore expand our pre-clinical work to Polθi studies in combination with RLT treatment. Using both alpha and beta emitting PSMA treatments, we will use relevant prostate cancer models to determine the impact of SHLD2 loss on Polθi-induced radiosensitisation both *in vitro* and *in vivo* prior to developing clinical trials in this area.

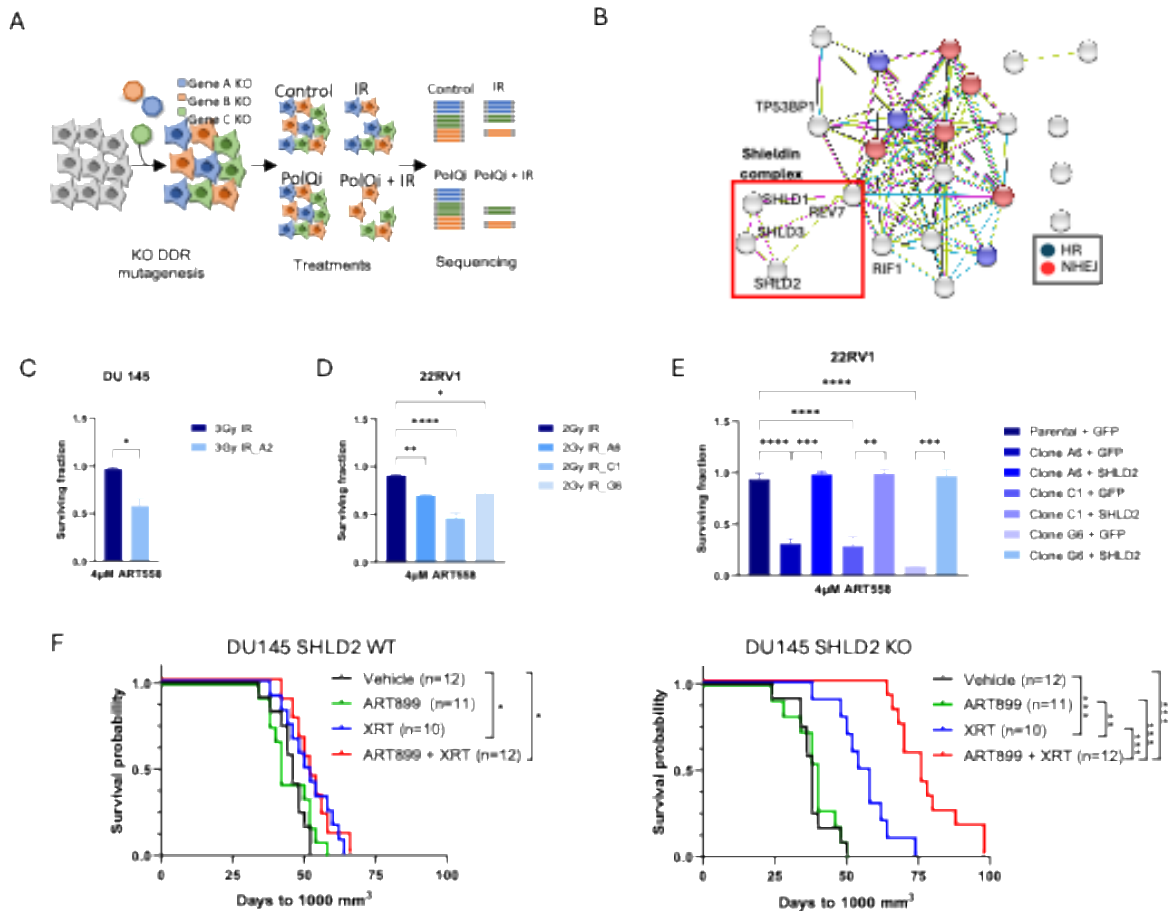

Figure 1. **(A)** Schematic of the DDR CRISPR screen in DLD1 cells. **(D)** Functional association networks of genes whose knockout caused significant radiopotentiation by Polθi in the DDR CRISPR screen (BioGrid) **(C)** Surviving fractions from colony formation assays of DU145 SHLD2 WT (parental) *vs* KO (clone A2) prostate cancer cells, treated with RT (3 x 2 Gy) and/or Polθi ART558. **(D)** Surviving fractions from colony formation assays of 22RV1 SHLD2 WT (parental), SHLD2 KO (clones A6, C1 and G6). **(E)** SHLD2 KO clones with reconstituted SHLD2 (+SHLD2) prostate cancer cells, treated with RT (3 x 1 Gy) and Polθi ART558. **(F)** Kaplan-Meier curves of DU145 SHLD and KO (clone A6) xenografts in NRG mice treated with 4 x 2 Gy and/or the Polθi ART899 (150 mg/kg BID for 14 days). *p<0.05; **p<0.005; *** p<0.0005 ; ****p<0.0001 Log–rank).

Translational potential of the project

By understanding the mechanisms by which Polθ inhibitors induce radiosensitisation we will be better able to design clinical trials stratifying those patients likely to benefit from Polθ treatment. The RLT aspects are of direct translational potential. Demonstrating that SHLD2 deficient tumours are more sensitive to RLT and are rendered more radiosensitive by Polθ inhibition would enable us to translate these findings into clinical studies of FDA-approved PSMA therapies including at earlier stages of disease.

Training opportunities

In addition to gaining exposure to routine tissue culture techniques and specialist DNA repair assays, the student will also be able to receive training in radioligand studies and murine xenograft experiments. They can also collaborate with our industry partners (such as Artios Pharma) and observe their approach to commercial drug development.

The ideal student background: The student is required to have experience in biological/medical sciences or medicine and an interest in radiotherapy/radioligand therapy and/or drug development.

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17. Leveraging the metabolic vulnerabilities of macrophages in pancreatic cancer- Keaton Jones

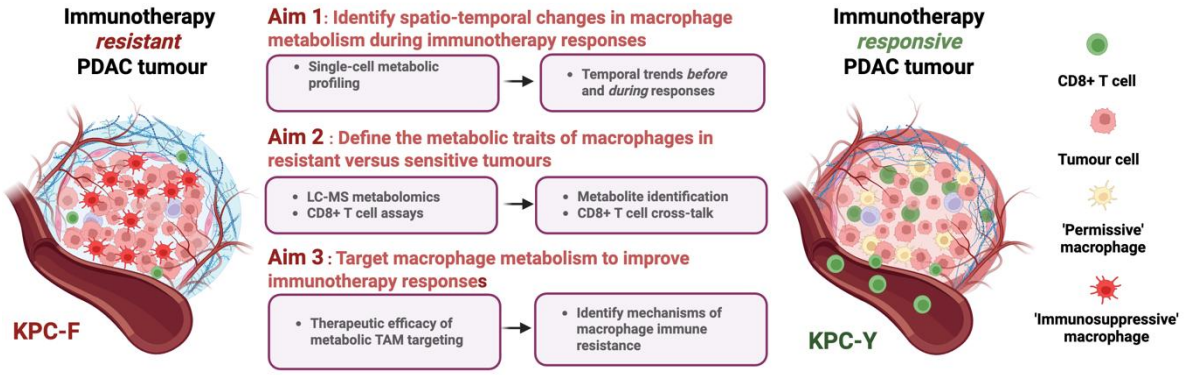
Primary Supervisor: Keaton Jones
Additional Supervisors: Eric O'Neil
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract of the project
Pancreatic cancer is one of the deadliest malignancies and remains largely resistant to immunotherapy. A major contributor to this resistance is the presence of tumour-associated macrophages (TAMs), which can suppress immune responses and promote tumour growth. Emerging research shows that the metabolic activity of these macrophages plays a critical role in shaping their function. However, how macrophage metabolism influences treatment response in pancreatic cancer remains poorly understood.

This project aims to investigate the metabolic landscape of macrophages in pancreatic tumours that either respond or do not respond to immunotherapy. Using advanced single-cell technologies and imaging techniques, the metabolic profiles of macrophages in both mouse models and human samples will be characterised. The metabolic traits that support immune suppression or stimulation will be identified, and we will explore how these can be targeted to enhance immunotherapy responses in pancreatic cancer. Ultimately, this work seeks to uncover new therapeutic strategies that reprogram the tumour microenvironment and improve patient outcomes.

Research objectives and proposed outcomes
Identify how macrophage metabolism changes during immunotherapy response.

1. Determine which metabolic traits promote immune suppression or activation.
2. Test whether targeting specific metabolic pathways in macrophages improves immunotherapy responses.



Academic value
This project builds on new insights into immunometabolism to uncover how metabolic cues influence immune responses in cancer. It will generate foundational knowledge of macrophage biology in the tumour microenvironment (TME), with relevance across multiple cancer types.

Translational potential of the project
Pancreatic cancer is notoriously difficult to treat, with minimal benefits from current immunotherapies. By understanding how macrophages influence treatment outcomes through their metabolic activity, this project aims to identify new therapeutic targets. Promising pathways such as creatine metabolism and PI3Kγ inhibition are already being explored in early-stage drug development. If successful, this project could lay the groundwork for clinical trials combining metabolic reprogramming agents with immunotherapy in pancreatic cancer.

Training Opportunities
The student will gain hands-on training in:

- Preclinical animal studies, Home Office Personal Licence A/B/C training provided.
- Single-cell metabolic and proteomic profiling **(**CyTOF, scMEP, IMC) with opportunities to attend hand on data analysis courses within the Medical Sciences Division.
- Functional immunology assays**,** including T cell cytotoxicity, antigen presentation, and macrophage-T cell co-cultures.
- Translational human tissue analysis using fresh pancreatic cancer resections.

The student will work in a multidisciplinary team combining immunology, oncology, metabolism, and clinical science, with access to national facilities and a strong track record of mentorship and career development.

Ideal student background: This exciting project is ideally suited to both clinicians and non-clinicians wishing to gain significant experience in cancer immunology. Students should have a background in biological sciences with some basic laboratory experience

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18. Modeling stromal inflammation and interactions in high-risk paediatric acute lymphoblastic leukaemia using bone marrow organoids – Abdullah Khan

Primary Supervisor: Abdullah Khan
Additional Supervisors: Anindita Roy
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract of the project

Despite improved survival in paediatric acute lymphoblastic leukaemia (ALL), outcomes for children with high-risk (HR) molecular subtypes remain poor, particularly those with early relapse or refractory disease. The bone marrow (BM) microenvironment plays a key role in leukaemia persistence, mediating immune evasion, therapy resistance, and inflammation. However, the precise mechanisms by which HR-ALL remodels and exploits the BM niche remain unclear, especially in the paediatric setting. We have recently developed a novel human bone marrow organoid (BMO) model that recreates key elements of the paediatric BM, including stromal, endothelial, and immune compartments[1,2]. This project will use BMOs engrafted with primary HR-paediatric ALL samples to systematically model stromal interactions, inflammation, and niche remodelling, employing advanced imaging, cytokine profiling, and single-cell transcriptomics.

Research objectives and proposed outcomes

Aim 1: Model HR-paediatric ALL expansion and localisation in an engineered human bone marrow organoid niche (BMO) [Collaboration: Dr Jana Koth]
We will engraft primary leukaemic blasts from two well-characterised HR-paediatric ALLs: KMT2Ar ALL, TCF3-HLF ALL onto Dr Khan's BMO platform and compare them to a standard-risk subtype: ETV6-RUNX1 ALL. Patient samples are already available in the Roy lab, and through the national VIVO Biobank. In addition, the Roy lab has developed human fetal HSPC derived models of both KMT2Ar[3] and TCF3-HLF ALL, which allows us to carry out this research even when patient material is limited, especially in the rare HR-ALL subtypes.

Co-cultures will be maintained for 2 weeks to allow dynamic stromal-leukaemia interactions to develop. Leukaemia burden and their effects on BMO populations will be assessed using spectral flow cytometry (panel to identify myeloid, lymphoid, and stromal cells). The spatial distribution and relationship with stromal and vascular elements will be assessed using 3D confocal microscopy and quantitative image analysis. Specifically, localisation to distinct niches (e.g. Osteoblastic, perivascular niches). To determine how HR-ALL remodels the stromal inflammatory landscape, we will collect cell culture supernatants at defined timepoints post-engraftment and perform OLINK assays. This will generate temporal secretory profiles unique to each patient sample, providing insights into niche activation, immunosuppressive signalling, and potential biomarkers.

Aim: 2 Map ALL-stromal immune crosstalk at single cell resolution
We will perform single-cell RNA sequencing (scRNA-seq) of the leukaemia engrafted organoids at key time points identified using data from Aim 1. Using cell-cell interaction modelling (CellChat, nichenet) and integration with imaging data, we will construct interaction networks between ALL cells and niche components, identifying key ligand-receptor pathways driving leukaemia persistence and stromal remodelling. This will provide the first high-resolution map of HR-ALL-niche interactions in a humanised, physiologically relevant model.

Aim: 3 Validation of novel therapeutics in an ALL-BMO model [Collaboration: Prof A Karadimitris]
The ALL-BMO system will be used to validate novel cellular therapies developed by the Roy/Karadimitris labs [4]. 'Off-the-shelf' bispecific CAR-iNKTs targeting CD19/CD133, have shown promising results with better efficacy than CAR-T cells in preclinical in vivo xenograft models against KMT2Ar ALL[5]. We will test these immunotherapies

in this human BMO pre-clinical model to assess the efficacy of the therapy in a native niche with differential leukaemia burdens.

Translational potential of the project

This project represents a novel approach to modelling HR-paediatric ALL within an organoid system that faithfully replicates the human BM niche. By elucidating the inflammatory and stromal mechanisms supporting ALL persistence, this work will reveal new therapeutic targets and inform strategies to disrupt niche support in HR-ALL. Furthermore, patient-specific inflammatory signatures may serve as predictive biomarkers of therapy response or relapse risk, enabling more personalised treatment strategies for these high-risk patients.

Training opportunities

The student will be embedded within the Khan and Roy labs at the WIMM, benefiting from expertise in leukaemia biology, organoid technology, imaging, and computational biology. They will receive comprehensive training in BMO culture and engraftment, advanced microscopy, cytokine profiling, scRNA-seq, and cell-cell interaction modelling. The project provides a unique translational opportunity to work with primary patient samples, immunotherapy, develop expertise in complex multiomic data integration, and present findings at national and international conferences.

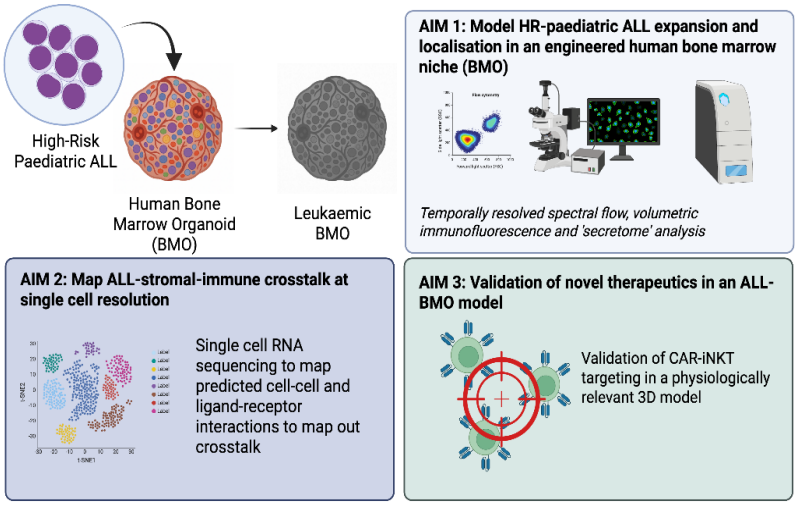

Figure 1: Schematic outline of proposed project. 3 aims developing and leveraging a high risk-paediatric ALL engrafted organoid approach that enables the detection of novel biomarkers, mechanisms, and therapeutic routes.**Outcome:** Integrated multiomic and imaging datasets will define key inflammatory pathways, niche remodeling signatures, and actionable targets driving HR-ALL persistence within the BM microenvironment.

Ideal student background: This project would be suitable for a medical science, biochemistry/biology undergraduate or master's student who wants to explore the fundamentals of leukaemia biology to improve patient outcomes. It is also suitable for clinicians because of the direct relevance to patient care and potential translational benefits. To deliver the project we are seeking a motivated and curious undergraduate/master's student, who has an understanding of biological processes. We are particularly keen to support students/clinicians who want to pursue a career in cancer research, as this project will provide a broad skill base to build upon for a future independent research career. Previous lab experience, particularly in cell culture, would be beneficial, but is not essential.

References

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19. Dietary modification to augment colorectal cancer treatment – Dimitrios Koutoukidis

Primary Supervisor: Dimitrios Koutoukidis
Additional Supervisors: Simon Buczacki and Victoria Woodcock
Eligibility: Track 1 applicants are eligible to apply for this project

Abstract of the project

Obesity is associated with worse progression-free survival among cancer survivors. One of the reasons might be that patients with obesity are receiving lower doses of chemotherapy compared with patients without obesity. Preclinical models show that energy restriction reduces chemotherapy toxicity and may augment chemotherapy efficacy. This is hypothesised to be driven by improvements in glucose regulation and reductions in IGF-1 through stress resistance in healthy cells and stress sensitisation in cancer cells. Furthermore, they suggest that standalone energy restriction is not as efficacious as a combination of energy restriction and established cancer treatments, such as chemotherapy.[1]

Although fasting itself around chemotherapy seems to not raise safety concerns based on a limited number of clinical cases and has shown some preliminary potential for increasing chemotherapy efficacy (higher radiological response and percentage of Miller Payne 4/5), adherence is challenging, the employed protocols vary, and the substantial increase in energy intake for 2-3-week period following the short-term (24-60h) fasting period during chemotherapy administration may negate its benefits. Furthermore, the 24-60h fasting that has shown benefit in mice cannot be expected to have the same effect as a 24-60h fast in humans, given the different physiology and lifespan. Instead, it is likely that a longer period of energy restriction will be required to achieve results of similar magnitude. Additionally, intentional weight loss may be considered counterintuitive during cancer treaatmtent as weight loss, primarily muscle mass loss, has been associated with worse outcomes.[2 3]

Existing low-energy dietary interventions in other clinical settings have been shown to affect the postulated mechanism. Intentional weight loss among people with obesity in other clinical settings has reliably improved glucose regulation and reduced insulin with the relationship following a dose-response manner and substantial weight loss to be required for a clinically meaningful improvement in glucose regulation to be observed. We and others have shown high adherence and significant weight loss with 800kcal/day diets including high-protein nutritionally replete total diet replacement with soups and shakes and food-based low-carbohydrate diets.[4-7]

This DPhil project will aim to adapt one such exisitng intervention, test its feasibility in a randomised controlled trial, and explore the mechanisms through which the intervention may increase chemotherapy efficacy.

Research objectives and proposed outcomes

WP1: Adapting the intervention to the setting.
Using the established Intervention Mapping approach and the MRC framework for complex interventions, together with involvement of relevant patient representatives, clinicians, and researchers. Through an iterative process, WP1 will focus on adapting an existing feasible and effective total diet replacement intervention.

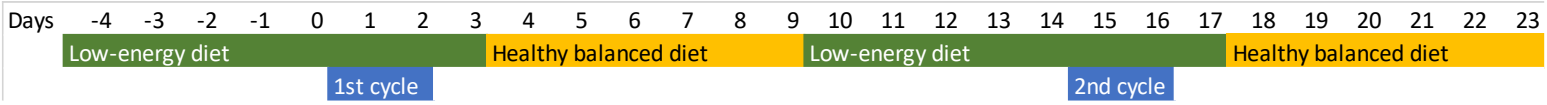
WP2: Testing the feasibility of the intervention

WP2 will involve running a randomised controlled trial to test the feasibility of the intervention. Specifically, the feasibility will be judged against pre-determined criteria on

- recruitment
- adherence to the diet
- retention at the last follow-up
- experience of participants following the intervention.

Population: Patients with obesity (BMI ≥ 30kg/m^2) and metastatic colorectal cancer.
Intervention: The exact nature of the intervention will depend on the findings on WP1, but we envisage that it involve a low-energy (800kcal/day) intervention through total diet replacement or through food-based low-carb diet from 5 days before each chemotherapy cycle to 1 day after each cycle followed by advice for a healthy balanced diet for the remaining period.

***Figure 1**: Proposed diet for the first 2 cycles of chemotherapy by day. Green indicates low-energy diet Orange indicates healthy balanced diet, and blue indicates days of chemotherapy.*



Comparator: Standard care (chemotherapy and existing CRUK leaflet on diet during treatment).
Sample size: With 72 patients (36 in each group), the trial will be 90% powered at one-sided 5% level based on the normal approximation approach to detect whether the proportions for adherence and retention criteria are truly above 35% and 65%, respectively, based on an alternative hypothesis that they will be above 60% and 85%, respectively.
Feasibility outcomes: These will include rates of recruitment, adherence, and retention.
Process outcomes: Experience and acceptability of the intervention based on semi-structured qualitative interviews with intervention participants.
Exploratory outcomes: Relative dose intensity, toxicity, and tolerance; and health-related quality of life.

WP3: Exploring the mechanisms of the intervention
The objectives of this WP is to explore potential mechanisms through which the intervention may increase chemotherapy efficacy.
Process: Participants will be asked to provide blood and urine samples before and after each cycle of the low-energy diet and each cycle of chemotherapy. Samples will be analysed for

- inflammation and immune serum markers (using the Olink Explore384 and macc cytometry (CyTOF)). The Olink panel will enable quantification of 368 low-abundance inflammatory cytokines and soluble immune mediators and CyTOF will enable quantification and functional assessment of >50 distinct immune cell subsets.
- urine and serum metabolomics and proteomicsmTOR & pmTOR PBMC ELISA.
- **Translational potential of the project.** Describe the relevance of the project to cancer research or patient care.

Developing novel therapeutics that are safe and effective with minimal side effects is a key priority for both cancer research and patient care. This need is highest among patients with metastatic disease, which this DPhil targets. This project will pave the way for a definitive clinical trial that will assess the effectiveness of this intervention in improving response to chemotherapy. If successful, this is likely to reduce the burden for patients and increase their progression-free survival.

Training opportunities
The student will have the opportunity to be trained in an array of different methodologies, including the development of complex interventions, recruiting to, managing, and analysis the results of a randomised clinical trial, qualitative analysis of interviews, laboratory techniques, and analysing of complex large-scale

biomarker data. This will include targeted formal courses within and outside Oxford as well as on-the-job training. They will have access to standard operating procedures from a relevant clinical trials unit, weekly meetings with supervisors, and access to a diverse pool of researchers applying the above methodologies at both NDPCHS and NDS.

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20. Vaccination to prevent progression of Monoclonal B-cell Lymphocytosis (MBL)/Stage A Chronic Lymphocytic Leukaemia (CLL) to treatment requirement – Carol Leung

Primary Supervisor: Carol Leung
Additional Supervisors: Eleni Adamopoulou, Pauline Robbe
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract of the project
Chronic Lymphocytic Leukaemia (CLL) is the most common adult B-cell malignancy in the western world. It typically evolves from a precursor state known as Monoclonal B-cell Lymphocytosis (MBL), which is prevalent in older adults. While many individuals with MBL and early-stage CLL remain asymptomatic, approximately two-thirds eventually progress to symptomatic CLL requiring treatment. Currently, there are no clinical interventions to prevent this progression. Treatment of later-stage CLL is rarely curative and often requires long-term therapy associated with side effects and decreased quality of life (1). The OxPLoreD study (Oxford Pre-Cancerous Lymphoproliferative Disorders: Analysis and Interception study) is an Oxford University led study (Schuh) which recruited 500 participants with MBL/Stage A CLL. The primary goal of this study is "to identify the clinical, genomic and immunological predictive markers of progression to malignant disease". This study has identified distinct biological and clinical subgroups within CLL (2), highlighting the heterogeneity of the disease and reinforcing the need for early, stratified intervention strategies. Building on this previous work, this project aims to develop a preventative vaccine to halt MBL/CLL progression. Leveraging data from the OxPLoreD study, we will identify CLL-specific neoepitopes by integrating RNA sequencing (RNAseq) with existing whole-genome sequencing (WGS) data. We will prioritise and validate candidate neoepitopes for their potential to stimulate anti-tumour immunity, generate vaccine constructs, and assess immunogenicity in preclinical models. This work will lay the foundation for future clinical trials targeting MBL/Stage A CLL patients.

Research objectives and proposed outcomes
1. Identify neoepitopes using different bioinformatics tools. From the OxPLoreD study, we have access to paired WGS and RNA-seq data derived from 53 diagnostic samples of individuals with MBL/early-stage CLL. We will use this matched genomic and transcriptomic information to predict tumour-specific neoepitopes by integrating multiple bioinformatics tools, including NetMHC and PRIME, to assess peptide binding affinity to HLA alleles. Cross-validation between prediction algorithms will help enhance reliability and prioritise high-confidence candidates. The resulting epitope list will then be compared to 845 paired tumour-normal CLL genomes previously generated by us (2) to filter out those that are not CLL-specific, thereby ensuring disease specificity. The refined list will serve as the foundation for further experimental validation.
2. Prioritise neoepitope candidates and validate antigenicity. After obtaining the list of candidate neoepitopes, we will prioritise those with high predicted binding affinity and structural suitability. We will employ mass spectrometry-based immunopeptidomics using paired blood samples to confirm the natural processing and presentation of these neoepitopes on HLA molecules. This will be followed by functional validation experiments (IFN-γ ELISpot assays) to assess the ability of these neoepitopes to elicit specific T cell responses. Additional validation may include peptide-MHC tetramer staining and flow cytometry to evaluate T cell recognition and activation. These experiments will help ensure that only the most promising neoepitopes are advanced to the vaccine development stage.
3. Generate vaccines against validated neoepitopes and evaluate immunogenicity. Having the list of validated neoepitopes, we will design and generate the vaccines using the viral vector (3) and mRNA platforms. The immunogenicity of the developed vaccines will be evaluated using participant blood samples and appropriate animal models. Results will be correlated to presence or absence of the T-cell exhaustion signature in these patients that we have already identified from bulk T-cell RNAseq or single cell analysis.

Translational potential of the project
The predicted and validated neoepitopes identified in this study will be used to generate a vaccine specifically for individuals at high risk of progression from MBL or asymptomatic CLL. The goal is to halt disease progression before it requires treatment, potentially ending the current "watch and wait" approach and improving patient outcomes. Oxford has a strong track record in vaccine development, and the insights and data generated through this project are expected to lead to the first clinical trial of a preventative blood cancer vaccine in the UK. If successful, this would represent the first preventative immunotherapy for CLL, reducing reliance on long-term treatments and their associated side effects. Importantly, the strategy may be adaptable to other indolent haematological malignancies with well-defined precursor stages.

Training opportunities
This project is a novel collaboration between the Leung, Adamopoulou, and Schuh labs, offering unique and synergistic expertise that will provide the student a wide range of knowledge. Within the Leung lab, the student will receive comprehensive training in cancer immunology and vaccinology. This includes techniques such as in vitro culture of primary T cells, ELISpot assays, peptide-MHC tetramer staining, flow cytometry, and in vivo murine models to assess vaccine immunogenicity. The student will also be involved in vaccine construct design and optimisation using both mRNA and viral vector platforms. The student will also get Home Office Modular training to gain a Procedure Individual Licence for conducting animal research. The Adamopoulou lab offers students the opportunity to develop expertise in antigen processing and presentation in cancer. They will receive training in mass spectrometry-based immunopeptidomics approaches to investigate HLA-restricted presentation of CLL-specific immunopeptides (HLA ligands) on malignant cells. In the Schuh group, the student will get specialised training in bioinformatics from Dr Pauline Robbe, and learn neoepitope prediction from WGS and RNA-seq data analyses, and computational modelling of HLA binding. This project will offer a rich, multidisciplinary learning environment, equipping the student with both experimental and computational expertise relevant to translational cancer research.

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

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21. Identifying and targeting metabolic vulnerabilities associated with isocitrate dehydrogenase mutations in acute myeloid leukaemia and glioma – James McCullagh

Primary Supervisor: James McCullagh
Additional Supervisors: Ian Tomlinson
Eligibility: Track 1 applicants are eligible to apply for this project

Abstract of the project
Cancer cells undergo significant metabolic reprogramming to support rapid growth and proliferation. The most frequently mutated genes directly involved in cancer metabolism are those encoding the isocitrate dehydrogenase enzymes (IDH 1 & 2) [1-3]. These have been identified in over 20 different tumour types but most research has focussed on acute myeloid leukaemia (AML) (>40% have mutations) and low-grade glioma (>70% have IDH mutations)[4-6]. It is now well established that somatic *IDH1 & IDH2* mutations catalyse the neomorphic reduction of 2-OG to 2-hydroxyglutarate (2-HG) [7,8]. Elevated cellular R-2-HG in cancer cells has been proposed to inhibit enzymes that regulate transcription and metabolism, leading to effects linked to epigenetic, cytoplasmic and mitochondrial processes (Figure 1). 2-HG may be involved in tumour development via altered gene expression and epigenetic reprogramming and therefore also serve as a cancer signalling molecule by infiltrating the tumour microenvironment and influencing neighbouring cells. For example, both 2-HG (and succinate another oncometabolite) have been shown to inhibit 2-oxoglutarate dependent oxygenases that catalyse chromatin modifications (the JmjC histone demethylases, TET oxygenases) and the HIF prolyl hydroxylases which are involved in the hypoxic response. Despite these insights, a systematic understanding of how 2-HG contributes to tumorigenesis is lacking, holding back the development of novel targeted therapeutics. Small molecule inhibitors of mutant IDH enzymes have been developed and a mutant IDH1 inhibitor (*ivosidenib*) has been approved for clinical use with acute myeloid leukaemia (AML)[9,10,11,12], with preliminary antitumour activity shown in other cancers [13,14,15,16]. However, *ivosidenib* resistance has emerged as a consequence of a second-site mutations producing IDH1 R132C/S280F [17-19] or IDH1 R132C/D279N[20], or acquisition of oncogenic IDH2 mutation[20]. This resistance limits effectiveness of these drugs in the clinic and patients with IDH mutant cancers currently face limited treatment options.

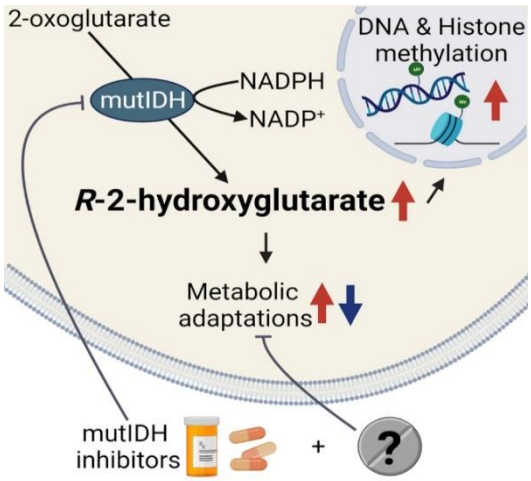

Figure 1: Mutations in IDH enzymes are commonly found in acute myeloid leukaemia and glioma, but their role in tumorigenesis is not well understood. A better understanding of how these mutations affect cellular function has the potential to reveal novel targets and help combat resistance to the current generation of IDH inhibitors.

New chemotherapeutic approaches are therefore needed for the treatment of AML patients, and a broad range of cancers expressing IDH mutantations.[21,22] The overall aim of this project is to comprehensively investigate altered biochemical mechanisms associated with IDH mutations in cancer cells promoting tumorigenesis/cancer progression and to target these vulnerabilities with novel inhibitors as alternative or combination therapeutic targets.

Research objectives and proposed outcomes
We will perform comprehensive multiomic analysis using a range of IDH mutant and wild-type cancer cells +/- IDH inhibitors and selected chemotherapeutics/exploratory compounds to dissect 2-HG-specific effects at the metabolome, proteome, transcriptome and epigenome levels. By stitching together data from these various 'omic' layers we will build up a system-level overview of mutIDH-specific functional changes in cellular processes and use this to identify potential drug and inhibitor targets.

Specific objectives of the project will be to

- Use generic engineering to generate and validate cellular models harbouring specific IDH mutations (Tomlinson Group).
- Perform multiomic measurements on cells +/- IDH mutations to identify metabolic, proteomic, transcriptomic and epigenetic effects specifically associated with IDH mutations (both groups).
- Dissect the effects of elevated 2-HG on identified targets using +/- IDH inhibitors and experimental therapeutics (McCullagh Group).
- Perform secondary screens to validate and explore the primary hits involving cell inhibition studies using synthetic inhibitors of IDH variant and wildtype (All clinically explored compounds and many others are available, importantly including the first wildtype IDH1/2 inhibitors) results will be used to design improved inhibitors, including to overcome the resistance to current IDH mutant inhibitors.
- Perform mechanistic studies exploring gene transcription and epigenome effects on downstream metabolic phenotypes including using metabolic isotope tracer to validate mechanistic insights (McCullagh and Tomlinson Groups).

Translational potential of the project
The results will provide key information on the impact of IDH mutations on cellular function and generate novel targets. This will inform three areas with important translational potential: i) Establish multimodal novel therapeutic targets for early cancer detection, potentially relevant across multiple cancers which harbour IDH mutations, in particular glioma and AML. ii) Identify potential new diagnostic biomarkers that could be exploited to guide therapy. iii) Provide the basis for mechanistic insights into common metabolic changes linked to tumorigenesis and cancer progression with utility as molecular signatures for diagnosis and prognosis. This work will form the basis for a larger scale program funding application to CRUK for studies on cancer metabolism focussing on development of novel therapeutics for IDH mutant cancers.

Training opportunities
Depending on the interests of the student, the project will provide experience in fields including genetic engineering, chemical biology, 'omic' sciences and analysis of large datasets using a wide range of bioinformatic tools. Methods that may be used include:

- CRISPR-Cas9 genetic engineering
- Hands on practical metabolomics and proteomics by mass spectrometry
- Mulitomic data analysis and data integration including univariate and multivariate data modelling and pathways and network analysis.
- Cell Biology methods including tissue culture, protein production
- Cell viability and drug monitoring assays and inhibitor design and medicinal chemistry.

Ideal student background: We welcome students with an interest in applying multiomic approaches to understand cancer biology. The focus will be on fundamental science-pathology interface. An interest in applying instrumental methods to investigate well-controlled cellular systems is welcomed. We expect the student's ideas and interests to help shape the direction of the research.

References

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22. A new target to treat ALT-dependent tumours – Peter J. McHugh

Primary Supervisor: Peter J. McHugh
Additional Supervisors: Christopher J. Schofield
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract of the project
Telomeres are the DNA structures that protect the ends of chromosomes. Cancer cells invariably activate mechanisms required to maintain the length and therefore integrity of these structures as part of their conversion into immortal cells with limitless potential to replicate and metastasise. Most tumours achieve this by upregulating an enzyme called telomerase, but a significant minority of tumours activate a pathway call 'ALT' to achieve this aim. Importantly, many tumours that particularly affect children and young people (sarcomas, central nervous system tumours) often rely on the ALT pathway (1). These tumours have a particularly poor prognosis, and so strategies to target ALT-dependent tumours are badly needed (1). A recently identified key player in the ALT pathway is the SNM1A DNA repair nuclease. We propose a project to understand the role of SNM1A in ALT, and pioneer methods to inhibit SNM1A as a strategy to selectively target ALT-dependent cancers.

Research objectives and proposed outcomes
A number of strategies are being pursued in academia and industry to target ALT-dependent tumours, including targeting some of the proteins (enzymes) that are required for the ALT pathway. In reality, the ALT pathway is complex, and many factors that are important for maintaining genome stability more generally are also needed for ALT. Our laboratories have a long-standing interest in a factor called SNM1A (encoded by the DCLRE1A gene). We have pioneered the biochemistry and cellular characterisation of this factor in DNA repair (2, 3, 4), solved its structure (5) and have also worked to generate the first small molecule inhibitors of SNM1A to inspire drug discovery efforts (6). Strikingly, in 2023 it was reported that SNM1A is important for a process involved in the ALT pathway known as 'break-induced DNA replication' (7). However, while performing an initial mechanistic study of SNM1A in this biochemical step of ALT, the authors did not definitively establish whether all ALT-dependent cancer cells depend upon SNM1A for their sustained proliferation and the mechanism by which loss of SNM1A induces ALT cancer cell death. If so, this would represent an important finding, meaning that SNM1A could be regarded as a potential and key therapeutic target in ALT-dependent tumours.

Therefore, the key objectives of the project are four-fold:

1. Are all ALT-dependent cancer cells reliant on SNM1A for their continued proliferation? This will be achieved by measuring the survival and proliferation (of SNM1A-depleted cancer cells that employ ALT-dependent or -independent mechanisms for telomere maintenance.

2. Is loss of SNM1A associated with the acquisition of hallmarks of telomere damage? If so, this would support a specific role for SNM1A in maintaining telomeres in ALT cancer cells. This will be achieved by monitoring accumulation of ALT-associated PML bodies (APBs), high levels of telomere sister chromatid exchanges and the accumulation of C-circles. These are hallmarks of dysfunction that can lead to the selective death of ALT-associated tumours.

3. How is SNM1A recruited to telomeres to excute break indiuced replication during the ALT process? Here, we will combine CRISPR-Cas9-based genetic screens and proteomic approaches to tease out the molecular mechanisms by which SNM1A participates in telomere duplication

4. Can we target SNM1A therapeutically to selectively kill ALT tumour cells? This aim will build upon our prgamme of structure guided inhibitor development. We have already obtained moderate potency SNM1A inhibitors (6), but the proposed work would provide an excellent platform for their validation and optimisation. Together, this work should provide a definitive answer as to whether SNM1A is an attractive target in ALT-dependent tumours. In the long run, it would allow us to adopt ALT-dependency as an indication for whether SNM1A inhibitors could be utilised. Such targeted therapy could benefit affected young people who currently have few treatment options.

Translational potential of the project
This proposal addresses a key priority of the Cancer Research UK and the Oxford Centre as it uses basic science to aid drug discover and explore and validate novel therapeutic approaches, one of the four priorities of the Centre.

Training opportunities
The precise training wil depend upon the interests and profile of the student, but will be interdisciplinary in nature, potentially involving: cell culture, genomic engineering (CRISPR-Cas9 and base/prime editing), large-scale screens, general molecular biology methods, DNA damage and repair assays, advanced microscopy, cell sorting methods, protein purification chemical biology, medicinal chemistry, modelling, protein science/enzyme inhibition, and biochemical assays. The student will also benefit from collaboration with Dr Anna Rose, a paediatric oncologist with special interest in ALT-cancers, allowing focus on clinical and translational aspects involved in treating ALT-dependent cancers in children and young adults.

Ideal student background: This would suit those with a degree in biochemistry, biomedical science, physiology, genetics, chemistry, preclinical medicine or related discipline.

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23. Origins and selection of genetic instability in blast phase myeloproliferative neoplasms (BPMPN) – Adam Mead

Primary Supervisor: Adam Mead
Additional Supervisors: Beth Psaila, Charlotte Brierley
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract of the project
Genomic instability is a hallmark of cancer, with ~80% of cancer genomes possessing large-scale alterations. The processes that enable genetically unstable clones to expand and become dominant remain poorly understood. Blast phase MPN (BPMPN) is a highly aggressive, treatment-resistant acute leukaemia subtype, marked by frequent *TP53* mutations and complex copy variants including chromothripsis, with typically a rapidly fatal disease course.[1,2] Conventional treatment approaches are ineffective, and few patients are cured by allogeneic stem cell transplant.[3,4] There is thus a major unmet need to identify novel treatments.

We have recently identified one route to genetic instability in BPMPN: recurrent chromothripsis on chromosome 21 ('chr21amp') amplifies *DYRK1A*, which both promotes genetic instability and cell survival.[4] DYRK1A represents a novel actionable therapeutic target which we plan to translate into Phase 1 studies, highlighting the power of deep interrogation of large primary patient datasets.

More widely, the triggers of genetic instability and selection mechanisms for specific rearrangements remain unknown. MPNs, with their long latency, defined hierarchy, and accessible samples, offer a unique model to study clonal evolution. Understanding the mechanisms underpinning the generation of, and the clonal advantage gained by genetically unstable clones may lead to novel therapeutic approaches for BPMPN - and more widely in myeloid disease, where *TP53* mutant disease lacks any effective treatment options.

Research objectives and proposed outcomes
We wish to understand the mechanisms by which genetic instability occurs in BPMPN and how clones are selected for survival in the bone marrow microenvironment, with the ultimate aim of identifying actionable therapeutic vulnerabilities.

Aim 1: Identification of recurrent drivers of genetic instability in primary patient samples progressing from chronic to blast phase MPN.
Over the past 7-10 years, with support from the Oxford Cancer Centre, we have collated circa 50 serial samples of peripheral blood/bone marrow mononuclear cells from patients with chronic phase (CP) MPN who subsequently progressed to blast phase (BP) with features of genomic instability, along with >400 samples from patients whose disease has not progressed to date. These have undergone extensive phenotyping, including whole genome sequencing via Genomics England, next generation sequencing myeloid panels, transcriptomic analysis, SNP arrays, and flow cytometric evaluation of the haematopoietic stem and progenitor compartment.

Aim 1 sets out to test the hypothesis that the processes that drive disease at progression are already identifiable at the CP stage, highlighting the opportunity for early intervention. The candidate would curate and complete the multiomic annotation of serial CP/BPMPN samples (clinical, mutation (panel or WGS/WES), copy number (SNP array or WGS), single cell assays (RNA and ATAC), disease trajectory analyses (SCIFER analysis of WGS data))[5] with the intent of investigating the origin of DNA damage, and the selection processes that enable the clone to survive the initiating event. Testable hypotheses will include 1) that the stem cell and mutation dynamics in patients who go on to transform are accelerated in CP patients who progress compared to those that do not[5] 2) those who progress demonstrate a predilection for the acquisition of DNA damage, potentially driven by proliferation-linked enrichment for replication errors[6,7], 3) that failure of immunosurveillance is key in enabling disease progression.[8]

Aim 2: Establishment and validation of a functional genomic screen in a murine model for drivers of genomic instability in MPN

Leveraging our prior work on the role of *DYRK1A*[4] in genetic instability in BPMPN, we have generated a triple knock-in mouse model that expresses *Trp53*R172H/+,*JAK2V617F/+* and overexpresses *Dyrk1a.* The *Trp53*R172H/+, *JAK2V617F/+* model is well-established and induces BP-MPN with 100-150 days latency. We hypothesise that overexpression of *Dyrk1a* will further promote genetic instability. We would cross our mouse model with BALB/c mice to enable haplotype phased SNP typing to detect copy number aberrations at high throughput and sensitivity as a readout. We propose using this model to deploy a targeted functional genomic screen - including genes/processes identified in **Aim 1** and those highlighted as regulators of chromothripsis in a recent CRISPR-Cas9 screen in a human cell line model[7] - to identify novel dependencies and evaluate whether their modulation promotes leukaemic progression in this murine model. Functional validation assays (flow cytometry characterisation of the HSPC compartment, *ex vivo* stem cell assays to evaluate clonality[9], and spectral karyotyping to assess genomic stability) are well-established in our laboratory[10].

Aim 3: Validation of candidate targets driving disease progression and genomic instability in BPMPN

Intersection of the genomic, transcriptomic and functional data ascertained across **Aims 1-2** will help filter out technical noise in the data and allow us to identify 3-5 candidate target genes/pathways. These targets will undergo functional validation in MPN cell lines, e.g. HEL, BaF3, SET-2 and organoid models using techniques established in the Mead laboratory. These studies will provide preliminary data to form the basis for future funding applications, including in vivo studies and development of new tool compounds.

Collaborations:

This project will be highly collaborative. Collaboration opportunities include with Genomics England (for the whole genome sequencing datasets), DNA damage experts at the MRC WIMM (Prof KJ Patel, Prof Peter McHugh, Prof Ross Chapman), and interactions with other centres in Oxford (Ludwig Institute: Marketa Tomkova).

Translational potential of the project

The aim of this project is to directly translate any discoveries made through to impact for patients. Our lab has a track record in the identification and pre-clinical development of novel targets in MPN.[11,12] Furthermore, the proposed programme may also lead to the identification of novel biomarkers to predict risk of BP-MPN and guide selection of therapy. By integrating the analysis of primary patient samples, advanced genetic mouse models and large publicly available datasets in the setting of MPN disease progression, we aim to identify tractable mechanisms of disease transformation that may be more broadly applicable in cancer, enabling opportunities for earlier intervention.

Training opportunities

The successful applicant will receive hands-on training in wet-lab molecular and cellular techniques, as well as advanced computational analysis. This includes expertise in high-parameter flow cytometry (including FACS sorting), tissue culture, single cell multi-omics, CRISPR/Cas9 genome editing, and advanced mouse genetics.

Candidates will have the opportunity to participate in the Oxford Biomedical Data Science Training Programme, which delivers formal training in the skills and methodology required for the analysis and interpretation of the large datasets. Students will be enrolled in the MRC Weatherall Institute of Molecular Medicine DPhil Course. This multi-day course is designed introduce students to a broad array of scientific techniques and theoretical principles.

Complementary skills training is provided through the Medical Sciences Division's Skills Training Programme, which offers courses in personal effectiveness, research management, and public engagement. The Department also operates a well-established mentoring scheme to support personal and professional development beyond formal supervision.

Ideal student background: The ideal candidate would have a keen interest in translational discovery science in haematology. We are keen to recruit a clinical haematology trainee with an interest in developing bioinformatic and computational skills, as the project requires the ability to access and assimilate large clinical datasets alongside molecular, genomic and mechanistic studies.

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24. Targeted delivery of drug-loaded oxygenated microbubbles for focal therapy of advanced prostate cancer – Ian Mills

Primary Supervisor: Ian Mills
Additional Supervisors: Fadi Issa, Pedro Durao, Annabell Roberti
Eligibility: Track 2 applicants are eligible to apply for this project

Abstract of the project

Prostate cancer (PC) is the most common male cancer in the United Kingdom and is one of the leading causes of cancer-related deaths [1, 2]. This high-incidence heterogenous multifocal disease has a long latency, in many instances through to metastatic progression in subset (typically 10-20%) of diagnosed cases. Enhanced activity of the androgen receptor (AR) is a major driver of PC, making anti-androgen therapy the most common treatment strategy. However, in the most advanced stages of the disease, known as castration-resistant prostate cancer (CRPC), tumour cells are insensitive to anti-androgen therapy and sustain pro-proliferative gene expression programs[3-5]. Importantly, AR promotes an immunologically cold tumour immune microenvironment (TIME), limiting the options of effective therapy, once CRPC becomes metastatic.Thus, having progressed, the disease is largely incurable[6].

In the absence of robust biomarkers that indicate metastatic progression risk when localised disease is diagnosed, it is challenging to intervene with systemic therapies due to toxicities and the risk of overtreatment. However, much progress has been made in identifying cell-surface markers of treatment-resistant cell types that correlate with poor prognosis pathology. The purpose of this project is to leverage these markers to exemplify targeted focal tumour treatment pre-clinically.
In addition, we know that hypoxia is also a feature of poor prognosis disease[7] and there may therefore be advantages to perturbing tissue oxygenation state at sites of cell-type targeted drug release.

We propose a first-in-field platform that couples **antibody-targeted, oxygen-loaded microbubbles** with **cyclin-dependent kinase-9 (CDK9) inhibitors**, releasing both precisely at ultrasound-defined foci. The strategy exploits (i) hypoxia as a driver of immune evasion, (ii) CDK9 inhibition to re-programme tumour transcription towards immunogenicity [8], and (iii) the clinical familiarity of microbubbles as imaging agents. By validating this approach in syngeneic and humanised mouse models, we aim to lay the groundwork for multi-target focal therapy that minimises systemic exposure while priming an anti-tumour immune response.

We will validate the cell-type targeting of the microbubbles in vitro using cell-lines engineered to overexpress the chosen cell surface markers that provide the capacity to target prostate adenocarcinoma (PSMA)[9], neuroendocrine prostate cancer (DLL3) [9] and both in association with cribriform pathology and stem-like cell states (B7-H3)[10].

RNA-seq and phospho- RNA polymerase II site analysis (Western blotting and proteomics). *In vivo* efficacy will be assessed through subcutaneous engraftment of a primary prostate cancer mouse cancer cell-line (Tp53 -/-;Pten -/-) known as DVL3, again engineered to express the three cell surface markers, alongside the unlabelled parental line [11, 12]. These lines will be used as allografts, permitting an assessment of systemic and site-specific immune responses to treatment and are being used as part of a project to study the systemic effects of CDK9 inhibition. Subsequently this will be extended into a humanised mouse model developed to support the engraftment of patient-derived organoids. We will quantify treatment-induced modification of the TIME by measuring (i) intratumoural $CD8^+$ T-cell infiltration and proliferation, (ii) the CD8:Treg ratio, (iii) activation markers such as granzyme B and IFN-γ in effector T cells, and (iv) depletion or functional impairment of myeloid-derived suppressor cells. We will use molecular profiling to assess STING and type-I interferon pathways, providing a mechanistic bridge between CDK9 inhibition, hypoxia reversal and adaptive immune priming.

Research objectives and proposed outcomes

The project brings together materials/engineering science (Stride group) with prostate cancer biology (Mills group) and the characterization of the immunological impact of interventions (Issa group) to develop a solution for the targeted delivery of immune modulatory drugs. No such solutions currently exist for the treatment of prostate cancer and are urgently needed because many drugs are highly effective in simple pre-clinical models, but induce significant systemic toxicities/immune side effects when translated to patients. Selectivity will be particularly beneficial for the treatment of prostate cancer, given that metastatic progression can occur over a significant time period post-diagnosis and local recurrence is a feature in cases treated with radiotherapy. We are exemplifying this with CDK9 inhibitors, building on a project to assess the systemic impact of these drugs on the TIME [8, 13-16]. The student will engage in both the drug formulation and the response characterisation gaining valuable experience of methodologies in all three groups aligned to the following objectives:

1. Manufacture of oxygen-containing microbubbles incorporating targeting antibodies on the microbubble surface – initially focussed on targeting PSMA and subsequently multi-targeting (DLL3 and B7-H4). Biophysical evaluation of microbubble recruitment to recombinant proteins and imaging to confirm microbubble integrity (Y1) – Eleanor Stride's group.
2. Incorporation of a CDK9 inhibitor(s) into the microbubbles and validation of incorporation and release kinetics (Y1) – Eleanor Stride's group.
3. Development of cell-lines overexpressing target cell surface proteins and fluorescently labelled (Y1) – Ian Mills' group – working with postdoctoral researchers Annabell Roberti and Pedro Durao.
4. In vitro assessment of selective binding and cytoxicity – imaging and cell viability assays (Y1) - Ian Mills'/Eleanor Stride's group – working with Annabell Roberti and Pedro Durao.
5. Subcutaneous engraftment of unlabelled overexpressing cell-lines into immune-competent mice – tumour volume/size measurements and baseline transcriptomic profiling (RNA-seq) and flow cytometry (immune markers) (Y2) – Ian Mills'/Fadi Issa's group - working with Annabell Roberti and Pedro Durao.
6. Microbubble delivery and ultrasound release – comparing the impact of injection of untargeted microbubbles to injection of targeted microbubbles with or without CDK9 inhibitor including piminidazole staining to define hypoxic regions (Y2 and Y3)- Ian Mills'/Fadi Issa's group - working with Annabell Roberti and Pedro Durao.
7. Extension to humanised mice engrafted with patient-derived organoids and haplotype-matched immune reconstitution to evaluate T-cell activation, check for off-target cytokine release, and assess durability of tumour control in a setting that mimics clinical heterogeneity including piminidazole staining to define hypoxic regions (Issa group – Y3).

Translational potential of the project

Clinically, PSMA imaging has been used to identify positive surgical margins in-theatre in patients undergoing radical prostatectomy (CRUK PROMOTE Trial, Oxford) [17]. Focal therapy in various forms (for example the Nanoknife) is also been trialled in patients in Oxford (PART Trial) [18] and London. Molecularly targeted focal therapy is however an approach that has yet to progress to clinical trials for treating prostate cancer because of the lack of pre-clinical evidence supporting drug delivery. Much work is underway, however, to develop antibody-drug conjugates for systemic administration,these conjugates require single-target (cell-surface and drug) combinations. Due to the multifocal molecular heterogeneity of the disease, more complete clearance of pro-metastatic prostate cancer clones using focal therapy in the prostate will require multi-targeting approaches. We envisage that this pre-clinical project will set the scene for adaptations to treatment, incorporating elements of the trials design in PART and tracer imaging. This willprovide an effective focal therapy alternative to surgery or radiotherapy for prostate cancer patients with high-risk disease at diagnosis or experiencing local recurrence following radical treatment [19]

Training opportunities

1. Cell-line culture and genetic manipulation of cell-lines (plasmid/CRISPR-Cas9)
2. Mouse models – animal handling, sub-cutaneous engraftment, drug administration and response monitoring
3. Immuno-staining and confocal microscopy
4. Spectral Flow Cytometry
5. Cell isolation from tissue samples and flow cytometry
6. RNA-seq and data analysis
7. Western blotting/SDS-PAGE
8. Real-time PCR
9. Nanobubble/microbubble preparation and characterization (for example optical microscopy, Interference Light Microscopy (ILM) and Laser Doppler Velocimetry)
10. Functionalisation of bubble preparations to prepare to bind to cell-surface targets (biotin-streptavidin-biotin bridging methodology as exemplified for anti-VCAM-1 targeting [20] or derivatization using Click Chemistry)
11. Preclinical ultrasound imaging and MRI

Ideal student background: This project lends itself to students with a background either in materials science or in biochemistry/cancer cell biology. From either background, there are training opportunities to bridge the gaps between these fields. We anticipate that the materials science component/drug delivery elements will form a core part of the first year of the project and applicants will need to have a strong interest in this area.

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25. Exploring the Role of Lipid Metabolism in Bone Metastasis and Radiation Sensitivity – Ejung Moon

Primary Supervisor: Ejung Moon
Additional Supervisors: Claire Edwards
Eligibility: Track 1 applicants are eligible to apply for this project

Abstract of the project
Bone metastasis is a destructive osteolytic bone disease that significantly impairs quality of life and presents therapeutic challenges. Alterations in lipid metabolism have been shown to impact tumour metastasis and radiation (RT) responses. RT is commonly used to manage symptoms and control localised disease in oligometastatic tumours, but balancing tumour control with normal tissue preservation remains a key challenge. RT-induced damage to healthy bone can lead to osteolysis, fractures, and pain. This study investigates how lipid-enriched bone microenvironments affect tumour metastasis and influence RT responses. By examining the role of the bone marrow microenvironment and its impact on radiation resistance, the study aims to establish a balance between tumour cell killing and normal tissue protection, ultimately enhancing RT outcomes for patients with bone metastasis.

Research objectives and proposed outcomes
Bone metastasis, a destructive osteolytic bone disease, significantly reduces quality of life and presents substantial therapeutic challenges[1]. Growing evidence suggests that alterations in lipid metabolism have a profound impact on tumour metastasis and radiation responses[2]. RT is commonly used as a key palliative treatment to alleviate symptoms and control localised disease in cases of oligometastatic tumours[3,4]. However, a persistent challenge in RT is balancing tumour control with the preservation of normal tissue, as RT-induced damage to healthy bone can lead to osteolysis, fractures, and pain. In this study, we aim to investigate how lipid enriched microenvironment affects tumour bone metastasis and examine ways to enhance tumour cell sensitivity to RT while minimising damage to normal bone tissue. Achieving a careful balance between tumour cell killing and normal tissue protection is crucial for the safe and effective application of RT[5,6].

Aim 1. To determine the impact of lipid enriched microenvironment on tumour metastasis.
Using both in vitro and in vivo systems, we will investigate how tumour metastatic behaviour and radiation responses are influenced by lipid enrichment. Co-culture experiments will be performed with bone metastatic breast cancer cell lines and adipocytes and/or bone cells. In vivo studies will be conducted using aged mice or by promoting bone marrow adiposity through a high-fat diet (HFD)[7]. Bone metastasis will be established using either the caudal artery injection method developed by the Moon lab or the intratibial injection approach developed by the Edwards lab[8]. For radiation, mice will undergo treatment using the small animal radiation research platform (SARRP), with image guidance to specifically target tumour cells. Dr. Hill at the Radiation Biophysics Core will provide expertise in radiation planning and execution. Tumour responses and bone destruction will be further monitored by tracking luciferase signalling, performing histology, and micro-CT imaging.
Aim 2: To identify specific lipid metabolic pathways that drive metastasis and radiation responses Building on previously acquired RNA sequencing data comparing parental and bone metastatic breast cancer cells, we will identify key candidate genes involved in lipid metabolism and metastasis. The expression of these genes and their associated proteins will be assessed in the co-culture system or through immunohistochemistry using tissues from the bone metastasis model. We will perform genetic or pharmacological inhibition of these candidate genes to determine whether their expression plays a critical role in regulating bone metastasis and radiation responses.

Aim 3: To determine the effect of FLASH RT on targeting bone metastasis while minimising normal bone destruction

In this aim, we will investigate whether FLASH RT can target bone metastatic tumours while protecting normal bone tissue from injury in the presence of bone marrow adiposity. FLASH RT is currently under clinical investigation for patients with bone metastasis, indicating its potential clinical efficacy[9,10]. Mice with bone tumours will receive radiation at both conventional and FLASH dose rates. The Petersson group will contribute their expertise in FLASH RT through their established radiation setup. Tumour cell killing and bone damage will be assessed using IVIS imaging, bone histology, and micro-CT.

Proposed Outcome:

- Providing insight into how lipid-enriched conditions influence tumour growth and radiation response.
- Identification of key lipid metabolic pathways that regulate bone metastasis and radiation sensitivity, offering novel therapeutic targets to improve cancer treatment outcomes.
- Demonstration that FLASH RT effectively targets bone metastatic tumours while minimising damage to normal bone tissue, suggesting its potential as a clinical treatment for patients with bone metastasis.

The joint supervision by Dr. Ejung Moon and Professor Claire Edwards will bring together expertise in breast cancer metastasis, radiation biology, and bone biology. Additionally, in collaboration with Dr Mark Hill and Dr. Kristoffer Petersson, students will gain exposure to the cutting-edge radiation techniques including SARRP and FLASH, which could help develop novel treatment strategies for bone cancer patients. This opportunity thus offers a multidisciplinary research experience, focusing not only on the biology of tumour metastasis but also on potential treatment strategies.

Translational potential of the project

In this project, we will develop a preclinical model of bone metastasis by inducing lipid enriched bone microenvironment. A better understanding of this interaction could provide critical insights into optimising RT, enhancing efficacy while minimising bone damage.

Additionally, we will investigate FLASH radiation, a novel technique delivering ultra-high dose rates, which is undergoing clinical evaluation for skin cancer and bone metastases. Our study will uniquely assess how FLASH radiation differentially impacts tumour and normal bone tissue within an adipocyte-rich bone marrow environment. Ultimately, this research could significantly improve the quality of life for patients suffering from immobility and pain due to bone metastases.

Training opportunities

Students will receive comprehensive training in both in vitro and in vivo experimental techniques. In addition to radiation procedures, they will gain hands-on experience in cell culture and molecular biology assays, including qRT-PCR, Western blotting, ELISA, transfection, and FACS analysis. They will also conduct co-culture experiments involving cancer cells, bone cells (osteoblasts and osteoclasts), and adipocytes. In vivo training will include mouse handling, tumour engraftment, and non-invasive imaging techniques. Students will further develop expertise in tissue processing and immunohistochemistry. Additionally, they will acquire skills in data analysis, critical thinking, and presentation, ensuring a well-rounded research experience.

Ideal student background: We are seeking a highly motivated and scientifically curious student with a strong foundation in basic molecular biology techniques. While previous experience in animal work is preferred, a keen willingness to learn in vivo techniques is equally valued. The ideal candidate will be a team player, eager to contribute to a highly collaborative and multidisciplinary research environment.

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26. Investigating the role of RAS activation in reprogramming the tumor microenvironment in colon cancer – Giulia Orlando

Primary Supervisor: Giulia Orlando
Additional Supervisors: Simon Buczacki
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract of the project
Colorectal cancer (CRC) development is driven by somatic mutations in genes within the RAS pathway such as *KRAS* and *BRAF*. Whereas RAS signalling has been shown to sustain tumour growth, effective targeted therapy to tackle RAS mutations directly, or its downstream targets, has proven very difficult to develop. Hence, there is a need to investigate RAS biology to unveil novel approaches that could be more broadly applied to RAS mutated tumours. The role for the tumour microenvironment (TME) in sustaining CRC progression and resistance to treatment is emerging, with the arise of cancer associated fibroblast (CAFs)(*1*, *2*). However very little is known about the direct role of KRAS activation in the early establishment of the TME. More specifically the epigenetic reprogramming of the stromal cells induced by *KRAS* mutation throughout a paracrine-mediated mechanism is poorly understood. To investigate the role of KRAS activation, colon organoid models will be developed using gene-editing CRISPR technology(*3*) to create *KRAS* mutation, and co-cultured with normal fibroblasts. Patient-derived organoid lines cultured with matched CAFs or normal fibroblasts will be established on selected pre-cancerous *KRAS*-mutated primary tissue from colon patients. We will implement a combination of multi-omics approaches(*4*) (RNAseq, ATACseq, 10X single-cell multi-omics) and study changes in the epigenetic landscape of stromal cells by profiling both the organoid models and primary tissue. Direct measuring of DNA methylation will be implemented using Oxford Nanopore Technology (ONT). Finally, organoid and *in vivo* models will be used to test promising selected candidates for functional validation. This study will provide novel insights into RAS signalling supporting the discovery of new treatments for CRC. Importantly, a better understanding of RAS biology has implications that go beyond CRC, as alterations in RAS pathway occur in the majority of cancers.

Research objectives and proposed outcomes
This project will investigate the direct role of KRAS activation in modifying the TME focusing on the early stages of CRC progression. We will profile organoid models and primary tissues in combination with cutting-edge multi-omics approaches to identify novel therapeutic target sustaining the TME epigenetic reprogramming. Additionally, the project will provide a framework to study how RAS activation in cancer cells has an active role in supporting the epigenetic remodelling of the TME. The models will be used to perform mechanistic studies and to characterise potential therapeutic opportunities.

Establishing a co-culturing system of KRAS-mutated CRC organoids and normal stromal cells. *Cancer* cells modify the surrounding cellular environment through paracrine mechanisms to support growth, vascularisation and progression to metastasis. To better understand this mechanism, we will implement a co-culturing model to investigate RAS-driven reprogramming of the TME. The models will give the opportunity to identify the epigenetics alterations driving stromal cells reprogramming and a novel understanding of the mechanisms that occur at the early stages of CRC development.

Identify transcription factors (TFs) and changes in DNA methylation driving the CAFs reprogramming. Transcriptomic studies have revealed significant differences in gene expression within cells in the TME compared to normal tissues. While aberrant TF activation has been reported, very little is known about the epigenetic mechanisms occurring at early stages of colon transformation. We will profile the organoid models and primary tissues using multi-omics cutting-edge technologies (RNAseq, ATACseq, ONT methylation, 10X single-cell multi-omics) leading to the generation of a unique epigenetic dataset to characterise early KRAS-dependent TME reprogramming. Computational methods will be applied to integrate the dataset and identify putative TF target for functional validation.

***Validate the KRAS-dependent molecular mechanism driving CAFs epigenetic reprogramming using the organoids and in vivo mouse models*.** We will use the co-culturing models and patient-derived organoids to assess the functional role of a selected set of TFs in regulating the epigenetic reprogramming of CAFs and assess their role in sustaining CRC growth. We will use primary samples to confirm the role in human disease development and *in vitro* and *in vivo* modelling to test therapeutic opportunities.

Translational potential of the project

CRC is the second most deadly cancer representing a significant global health priority. Advancements in CRC treatment have been limited with medical care involving a combination of surgery, radiotherapy and chemotherapy. Few targeted treatments have been implemented and often resistance occurs. The role of the TME has emerged as one of the causes of treatment failure. Very few therapies target the TME directly, hence the importance of the proposed project. This study will bring innovative treatment solution with the identification of novel druggable targets to reprogramme the TME to a normal stratus to disrupt tumour growth. Dual treatment targeting both the colon organoid with conventional therapies and newly identified TME-specific targets would also be tested *in vitro* and *in vivo*.

Training opportunities

The selected student will have opportunities to work on a multidisciplinary project and acquiring skills in both wet lab and computational analysis. The student will employ cutting-edge technologies in organoid modelling and culture, genomics (RNAseq/ATACseq, 10X single-cell multiome, ONT methylation) and genome editing (CRISPR editing), along with functional biology assays (FACS, imaging, viability assay, drug treatment). Training will be provided to acquire the relevant computational skills to analyse their own dataset independently. Opportunity to attend computational courses will be provided.

Ideal student background: While experience in either organoid modelling or genomics would be preferred, this project will be suitable for candidates with no prior experience, but a strong interest in developing multi-disciplinary skills. An interest in acquiring computational skills is highly desirable.

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27. Linear accelerator for Mega Voltage Photon FLASH radiotherapy – Krisoffer Petersson

Primary Supervisor: Kristoffer Petersson
Additional Supervisors: Geoff Higgins
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract of the project
Radiotherapy is an effective treatment for many cancers. Unfortunately, people treated with radiotherapy are at risk of significant side effects that can last a lifetime, including risks of other cancers and heart disease. This research will enable FLASH radiotherapy; a potentially revolutionary technology for the treatment of cancer that promises to cure more people and significantly reduce side effects.

Preclinical studies have shown that FLASH significantly reduces treatment side-effects. As a result, we could give up to one and a half times the usual radiation dose without any increase in normal side effects. This means that we can:

- Increase the dose we give and improve response to treatment
- Reduce the number of treatments sessions (fractions)
- Deliver the usual treatments with fewer side effects.

Also, FLASH radiotherapy means much shorter treatment times. This reduces the time that a patient has to stay in a fixed treatment position. Besides being much more comfortable for patients, it also reduces the risk of error that can happen with small changes in position. The reduced time also means that the volume of healthy tissues exposed to the radiation beam is reduced. This further lowers the risk and severity of side-effects.

Challenges
There are technical challenges that must be overcome before we can make this treatment available to patients. These challenges relate to producing Megavoltage (MV) photons at the high dose rates necessary for FLASH. Our proposal will help bring FLASH to patients. Our collaborator network offers a unique global partnership that combines the necessary expertise, experience and access to new technologies that are needed to resolve these technical challenges.

Research objectives and proposed outcomes
To date, most preclinical and all clinical FLASH research have been performed using proton or electron beams. However, there has only been a few clinical FLASH trials so far. These have been small (phase 1) trials looking at feasibility rather than exploring any clinical benefit with the technique. There are numerous limitations to deliver clinical FLASH employing these methods. Use of proton beams require large and expensive facilities, while FLASH using electrons is currently only capable of treating tumours that are close to the surface of the body. More than 95% of radiotherapy given at present uses MV photon beams at low dose rates.

I) trials looking at feasibility rather than exploring any clinical benefit with the technique. There are numerous limitations to deliver clinical FLASH employing these methods. Use of proton beams require large and expensive facilities, while FLASH using electrons is currently only capable of treating tumours that are close to the surface of the body. More than 95% of radiotherapy given at present uses MV photon beams at low dose rates.

We aim to develop ways to deliver MV photons at dose rates 1000x higher than standard radiotherapy. This will enable a FLASH-capable system to be produced at a cost comparable to that of existing clinical photon facilities, enabling world-wide deployment. This will allow FLASH to have a real impact on how radiotherapy is delivered.

Aim and approach
The aim of our proposal is to develop an accelerator and treatment technology for MV Photon FLASH treatments. We will achieve this by modifying and optimising standard components for FLASH delivery, that is, a short but very intense radiation beam delivery. Our proposal consists of five parts (work packages, WP):

WP 1 – Optimisation of the electron source (gun) and accelerating structure (waveguide)

WP 2 – Development of the (Radio Frequency) power source for electron acceleration,

WP 3 – Full accelerator assembly

WP 4 – Optimisation of photon beam production (target) and modulation

WP 5 - Performance measurements and evaluation of the final beam.

Translational potential of the project
If successful, this project will provide a future pathway to:

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

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

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

Training opportunities
Training on simulation software and practical construction of electron guns and accelerating waveguides for linear accelerators, with leading experts at the Department of Physics. The student will have the opportunity to train at Teledyne e2v, learning about RF-power sources and subsystems, and their implementation in radiotherapy. This general knowledge of RF-technology will be essential for the work carried out during the DPhil project. At the Department of Oncology, the student will earn about FLASH radiation biology and dosimetry from the members of the FLASH Radiation research group. Additionally, the electron FLASH linear accelerator available in the department will serve as a reference for all the work carried out throughout the project.

Ideal student background: The student should ideally have an MSc in Physics, Medical Physics, Biology, engineering, or other natural or medical science with a a significant interest in technology and its application in healthcare.

28. Microbubbles as drug carriers for patients with malignant pleural effusion – Najib M Rahman

Primary Supervisor: Najib M Rahman
Additional Supervisors: Nikolaos I Kanellakis
Eligibility: Track 1 and 2 applicants are eligible to apply for this project

Abstract of the project
Background: Malignant pleural effusion (MPE) occurs when fluid accumulates in the pleural cavity because of cancer. MPE is common, affecting 15% of all cancer patients with around 50,000 new cases diagnosed in the UK per year and bears a severe socioeconomic burden. In the USA alone the MPE-associated healthcare costs are around $1,900 million per year. Approximately, 90% of MPE occurs due to metastatic cancer from primary sites including lung, breast and colorectal. MPE is associated with poor prognosis which appears to be particularly pronounced compared to other metastatic sites, leading to a median survival time of 3 to 12 months from diagnosis. Large scale epidemiological studies demonstrate that even small volume MPEs are associated with poor prognosis, regardless of systemic treatments such as chemotherapy and immunotherapy. Current treatment for MPE focusses on symptom management (chest pain, breathlessness) treated with drainage procedures, but does not alter prognosis.

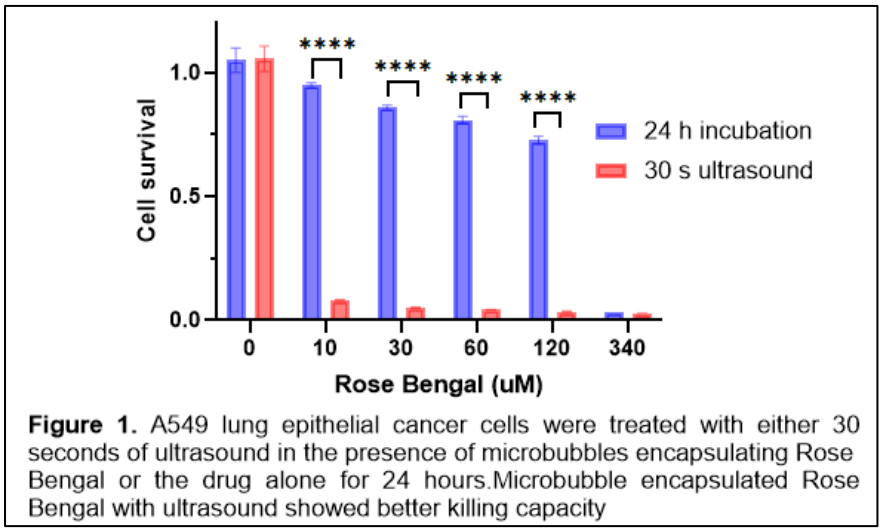

Figure 1. A549 lung epithelial cancer cells were treated with either 30 seconds of ultrasound in the presence of microbubbles encapsulating Rose Bengal or the drug alone for 24 hours.Microbubble encapsulated Rose Bengal with ultrasound showed better killing capacity

The current unmet clinical need: Taken together, these data indicate the need for the development of MPE specific therapies beyond systemic anticancer treatment.

Microbubbles could facilitate drug delivery: Coated gas microbubbles. originally developed as contrast agents for ultrasound imaging, have been re-engineered to carry small molecule drugs. By destroying the microbubbles using focused ultrasound at the target site highly localised drug delivery can be achieved.[1,2]. In addition, drugs which only become active when stimulated by ultrasound can be used to further reduce systemic toxicity. To assess whether this approach could be utilised to treat MPE, we exposed A549 lung epithelial cancer cells to either 30 seconds of ultrasound in the presence of microbubbles encapsulating an ultrasound sensitive drug (Rose Bengal), or the drug alone for 24 hours. Our preliminary data suggest that the microbubbles greatly increased cancer cell killing even over the much shorter exposure times (Figure 1). Given our unique access to the pleural space in vivo to injected drug delivery and ultrasound, this treatment modality offers a potentially exciting avenue for novel therapy.

Research Objectives and proposed outcomes
We have designed a translational study to evaluate the efficacy of the microbubble drug delivery methodology in MPE. We have a established a panel of patient derived MPE cell lines[3]. These cells are a faithful model of the human disease. We will expose these cell lines to different combinations of drugs, microbubbles and ultrasound. For these assays we will use the top five drugs as identified in a previous high throughput drug screening assay.[3] Cells will be cultured in 2D,3D, organoid, and we will assess viability and proliferation.

Research Objective 1: Identify the IC50 for different exposure conditions.
Research Objective 2: We will assess the cancer cell killing capacity and immune cell activation, when cancer cells are co-cultured with T cells.
Research Objective 3: Optimise the microbubble formulation encapsulating the top performing drug(s) to maximise drug loading and stability.
Research Objective 4: Evaluate the antitumour efficacy of drug-loaded microbubbles in an *in vivo* mouse model of MPE. For this the mice will receive injections of drugs encapsulated in microbubbles which will be activated with thoracic ultrasound. We will assess tumour control via ultrasound imaging, immunomodulation on the tumour microenvironment via immunohistochemistry, and and overall survival as previously done.[4,5]

Translational potential of the project
MPE is a significant clinical challenge, affecting approximately 30% of cancer patients. The incidence of MPE is increasing worldwide and currently there are no effective therapeutic treatments available. This project has a high translational potential. Microbubbles are widely used as ultrasound contrast agents and have been used in clinical trials for therapeutic applications.[6] A trial of drug-loaded microbubbles will also shortly take place in the UK for breast cancer. Our preliminary data show effective tumour cell killing and to the best of our knowledge this is the first time that microbubbles have been investigated as drug carriers in MPE. The use of fresh patient-derived MPE samples would allow us to phenotype the response and discover factors that limit the efficacy of the treatment. If the microbubbles demonstrate antitumour potential in our in vitro and ex vivo models we can proceed to a small scale local (Oxford University Hospitals, NHS Trust) Phase I (first in human) clinical trial to evaluate their safety and tolerability, as we have successfully done in the past[7].

Training opportunities
This is an interdisciplinary project between the Institute of Biomedical Engineering and CAMS Oxford Institute. The student would receive training on bubble manufacture, cell culture, flow cytometry, immunology, and statistical analysis. The student would benefit from engaging in an interdisciplinary environment that integrates bioengineering and biomedical sciences, providing exposure to a broad spectrum of research methodologies.

Ideal student background: Ideally, the student shall have some basic experience and understanding of cell culture and cancer biology.

References:
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29.Developing clinical algorithms for non-invasive myeloma monitoring using mass spectrometry and machine learning approaches – Karthik Ramasamy

Primary Supervisor: Karthik Ramasamy
Additional Supervisors: I-Jun Lau, Adam Cribbs
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract of the project
Monitoring minimal residual disease (MRD) in multiple myeloma (MM) is essential for guiding therapy and improving patient outcomes.(*1*) However, current methods for assessing deep responses to treatment rely heavily on bone marrow biopsies, which are invasive, painful, prone to sampling bias due to the patchy nature of disease and therefore unsuitable for frequent, longitudinal monitoring.(*2*)

Mass spectrometry (MS) offers a highly sensitive and specific approach for detecting tumour-derived monoclonal immunoglobulin (M-protein) in blood, offering superior resolution compared to currently available serological techniques.(*3*) As each patient's M-protein is unique, it serves as a specific, personalised biomarker for tracking tumour dynamics over time. MS assays can detect and quantify very low levels of M-protein, enabling more accurate and less invasive monitoring. Importantly, MS testing shows prognostic performance comparable to bone marrow-based MRD tests at 10^{-5} sensitivity(*4*) (recently approved as an early endpoint in myeloma clinical trials by the FDA), thus highlighting its potential as a robust, non-invasive alternative for long-term disease surveillance.

This project will validate high-resolution, M-protein quantification via advanced MS platforms for real-time assessment of tumour burden and residual disease. By modelling M-protein kinetics and applying machine learning-based predictive algorithms, we aim to enable accurate, frequent and individualised monitoring from blood. Ultimately, this approach could complement or replace bone marrow MRD testing with scalable, patient-friendly tools that support adaptive, precision-guided treatment.

Research objectives and proposed outcomes
This project aims to validate and implement high-resolution, non-invasive MS assays for monitoring tumour burden and treatment response in multiple myeloma (MM). Using well-annotated clinical samples from two major UK studies, the focus will be on assay benchmarking, modelling of treatment kinetics and clinical algorithm development.

- **RADAR** is a UK-wide phase II/III trial evaluating risk-adapted treatment intensification in newly diagnosed MM. It includes bone marrow MRD testing at 10^{-5} sensitivity, and provides paired blood and marrow samples for direct comparison of non-invasive MS assays with gold-standard MRD.(*5*)

- **MOSAIC** is a prospective observational cohort collecting serial blood samples from MM patients at multiple treatment stages, offering a real-world setting to evaluate assay performance, scalability and clinical utility.

Objective 1: Validate the analytical performance of commercially available mass spectrometry platforms for non-invasive M-protein quantification
This objective will benchmark the sensitivity, specificity and reproducibility of clinically available assays – such as EXENT QIP-MS (Quantitative Immunoprecipitation Mass Spectrometry), LC-MS (Liquid Chromatography Mass Spectrometry) and clonotypic peptide-based mass spectrometry (SEBIA M-inSight) – using paired samples from the RADAR trial. Assay performance will be compared against conventional serological tests and bone marrow

MRD (flow at 10^{-5} sensitivity) aiming to provide a validated blood-based monitoring platform with performance metrics suitable for clinical implementation.

Objective 2: Characterise M-protein kinetics and treatment response dynamics using longitudinal testing
Using serial blood samples from the RADAR and MOSAIC studies, the student will examine M-protein clearance patterns across treatment time points. Modelling of kinetic parameters will enable the identification of response trajectories and residual disease signatures, with the aim of improving relapse prediction, informing updates to current accepted response criteria (as defined by the International Myeloma Working Group, IMWG) and supporting the development of dynamic biomarkers to guide response-adaptive treatment decisions in the future.

Objective 3: Using computational modelling and machine learning, develop and evaluate predictive algorithms for response assessment and relapse detection
This objective will leverage longitudinal M-protein quantification data and associated clinical variables to train predictive models capable of classifying treatment response and forecasting relapse. Machine learning approaches – including regularised regression, ensemble methods and time-series models – will be used to capture complex, non-linear relationships between M-protein kinetics and clinical outcomes. Model development will prioritise interpretability and robustness, employing nested cross-validation and feature selection strategies to minimise overfitting. Trained models will be independently validated using held-out cohorts from the MOSAIC study and compatible external data sets (e.g. from Mayo Clinic, PETHEMa) and performance metrics (e.g., AUROC, sensitivity, specificity) will be benchmarked against conventional clinical predictors. The objective will also assess the feasibility of incorporating these models into prospective clinical workflows, focusing on scalability, clinical interpretability and decision-support utility.

Translational Potential of the Project
MRD is an increasingly important biomarker for risk stratification, treatment de-escalation and clinical trial endpoints in MM. However, bone marrow-based MRD assessment is logistically and clinically challenging for routine use due to its invasiveness and sampling limitations. This project will help deliver a scalable, patient-centred solution: non-invasive, high-resolution M-protein monitoring via MS platform/s. Demonstrating the clinical validity and utility of MS MRD using RADAR samples and real-world validation through MOSAIC samples, will facilitate its implementation in future clinical trials and potentially routine NHS care.

Training Opportunities
The student will receive comprehensive interdisciplinary training spanning advanced analytical, laboratory, computational and clinical domains. This will include hands-on experience with state-of-the-art mass spectrometry platforms, as well as immunoprecipitation workflows for high-resolution serum protein analysis. Training in computational modelling and data science will cover longitudinal data analysis, M-protein kinetic modelling and the application of machine learning for biomarker interpretation and relapse prediction. The student will also gain direct exposure to translational and clinical research through access to trial and cohort samples, insight into biobank governance and ethics, and collaboration with multidisciplinary teams across haematology and laboratory medicine. A key focus will be on developing personalised biomarker strategies, using patient-specific clonal M-proteins to enable precision diagnostics for minimal residual disease and support response-adaptive treatment. This diverse skill set will prepare the student for future leadership roles in translational cancer diagnostics and precision oncology.

Ideal student background: This project welcomes applicants from all academic backgrounds with a strong interest in translational cancer research. Ideal candidates may have training in biomedical sciences, medicine, molecular biology, bioinformatics, or related disciplines. No prior experience in mass spectrometry or machine learning is required; comprehensive interdisciplinary training will be provided. The project offers a unique opportunity to work at the interface of clinical haematology, analytical science and computational modelling,

contributing to precision medicine and early detection strategies. Motivated students with curiosity, critical thinking skills and a desire to impact patient care through innovative diagnostics are strongly encouraged to apply.

References

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30. Using wearable devices to investigate associations between sleep and circadian disruption, artificial light-at-night exposure and incident cancer risk – David Ray

Primary Supervisor: David Ray
Additional Supervisors: Rebecca Richmond
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract of the project
There is strong evidence from experimental systems that animals exposed to circadian disruption and alterations to the light-dark schedule exhibit immunosuppression, chronic inflammation, and cell proliferation, which are key carcinogenic characteristics [1]. Further, exposure to artificial light-at-night (ALAN), particularly blue wavelength light, has been found to inhibit nocturnal production of melatonin, which has been hypothesised to increase risk of cancer [2]. In humans, much of the evidence surrounding carcinogenic light-at-night exposure and circadian disruption is restricted to studies of night shift workers [3, 4]. While positive associations have been observed between night shift work and breast, prostate, colorectal cancer in particular, overall evidence is inconsistent.
The overarching aim of this DPhil is to fully appraise the role of sleep, circadian disruption and artificial light exposure in cancer. This will include deep phenotyping of sleep and circadian rhythm disruption (SCRD) and light measures from wearable devices obtained from large population-based studies, integrating cancer record data, harnessing genetic data to improve causal inference, and exploring high-dimensional molecular data to better understand mechanisms. With thorough and integrated approaches to data science being performed at scale, the project has the potential to further our understanding of cancer and to identify novel behavioural and therapeutic targets to reduce cancer risk.

Research objectives and proposed outcomes
The DPhil project will use data from wearable devices in two large-scale epidemiological cohort studies (UK Biobank and China Kadoorie Biobank) to investigate the links between i) objective sleep measures, ii) circadian parameters, iii) artificial light-at-night exposure (ALAN), and incident cancer. The research will draw on deep phenotyping of sleep, circadian rhythms and light exposure obtained from objective devices, extensive genomic and molecular datasets, as well as linked health data, to provide a step change in our understanding of the mechanisms underlying the links between sleep and circadian disruption (SCRD), ALAN and cancer risk in humans.

Specific aims are to:

1: Use a deep phenotyping approach to derive objectively-measured sleep, circadian rhythm and light measures from wearable devices in the UK Biobank and China Kadoorie Biobank.
2: Perform prospective epidemiological analyses to investigate associations between SCRD and ALAN in relation to cancer.
3: Conduct genetic analysis to identify novel variants influencing sleep behaviour, circadian rhythms and light sensitivity. This will build on previous genome-wide association studies (GWAS) (e.g. [5-8]) and gene-by-environment interaction studies (GWIS) [9] to provide insights into the genetic underpinnings of SCRD and circadian light sensitivity.
4: Leverage genetic, metabolomic and proteomic data to investigate molecular pathways linking SCRD, ALAN and cancer.
5: Use naturally occurring genetic variation encoding SCRD and light sensitivity (identified in aim 3) to uncover new therapeutic targets and preventative agents for cancer.

The student will work across the Big Data Institute (BDI), the Nuffield Department of Population Health (NDPH) and the Oxford Centre for Diabetes, Endocrinology and Metabolism (OCDEM) at the University of Oxford. They will be supported by supervisors with expertise in sleep and circadian medicine (Prof David Ray), genetic and molecular epidemiology (Dr Rebecca Richmond), biomedical informatics (Prof Aiden Doherty), and cancer epidemiology (Prof Ruth Travis). The supervisory team have ongoing collaborations and established profiles across sleep, cancer and wearables research, and all are involved in the NIHR Oxford Health Biomedical Research Centre "Better Sleep" theme. However, this DPhil project will represent a new collaborative effort investigating the role of sleep and circadian rhythm measures derived from wearable devices in relation to cancer.

Translational potential of the project

The results of this project have the potential to change the way we understand and prevent cancer by recognising its sleep and circadian underpinnings. By evaluating the role of sleep and circadian rhythms in cancer across different populations and subgroups, we can identify individuals most at risk of its adverse consequences and reduce health inequities in cancer incidence. This will inform targeted interventions and risk stratification efforts, for example with the incorporation of sleep and circadian rhythm disruption measures into cancer risk prediction models. If cancer populations are found to be differentially affected by sleep and circadian disruption, this will highlight the importance of improving sleep among patients whose diagnosis and treatment regime could further compound sleep problems. By probing the biological pathways underlying the links between sleep, circadian disruption, ALAN and cancer, this could provide fundamental biological insights as well as uncover novel therapeutic targets and preventative agents for cancer. This has the potential to inform therapeutic innovations and future clinical trials.

Training opportunities

The proposed project offers an exciting opportunity for the student to develop skills in epidemiology, genomics and biomedical informatics, with the potential to conduct prospective epidemiological analysis, genetic analysis, causal inference and machine learning approaches. We would encourage the student to attend a number of internal and external training courses to develop these skills, including:

- NDPH short courses in "Introduction to Epidemiology", "Practical Statistics for Epidemiology using R", "Practical Design of Epidemiological Studies", "Fundamentals of Statistical Software and Analysis"
- Short course in "Machine Learning of Wearables in Large Scale Biomedical Studies" (Prof Doherty is Course Director)
- Oxford Online Programme in Sleep Medicine modules: "The Physiological Basis of Sleep", "Introduction to Sleep Medicine and Methodological Approaches", "Circadian Rhythm Disruption and Sleep"
- Bristol Medical School short courses in "Genetic Epidemiology", "Mendelian Randomization", "Molecular Epidemiology", "Reproducible Health Data Science" (Dr Richmond is Course Tutor).

The student will also develop collaborations with the Sleep and Circadian Neuroscience Institute (Prof Ray), the Oxford Health Biomedical Research Centre "Better Sleep" theme (Prof Ray, Dr Richmond), the BDI Wearables Groups (Prof Doherty), and the Cancer Epidemiology Unit (Prof Travis) at the University of Oxford, as well as the Cancer Research UK Integrative Cancer Epidemiology Programme and MRC Integrative Epidemiology Unit at the University of Bristol (Dr Richmond). These links offer opportunities for the student to present their work at different group meetings and engage in discussions with experts, fostering new insights and supporting the development of scientific papers and conference presentations.

Ideal student background: Applicants from both clinical and non-clinical backgrounds are welcome to apply. Ideally the student should have received training in medical statistics, biomedical science, bioinformatics and/or genetics, experience or an interest in programming, and a clear motivation to pursue research in cancer epidemiology and sleep/circadian medicine.

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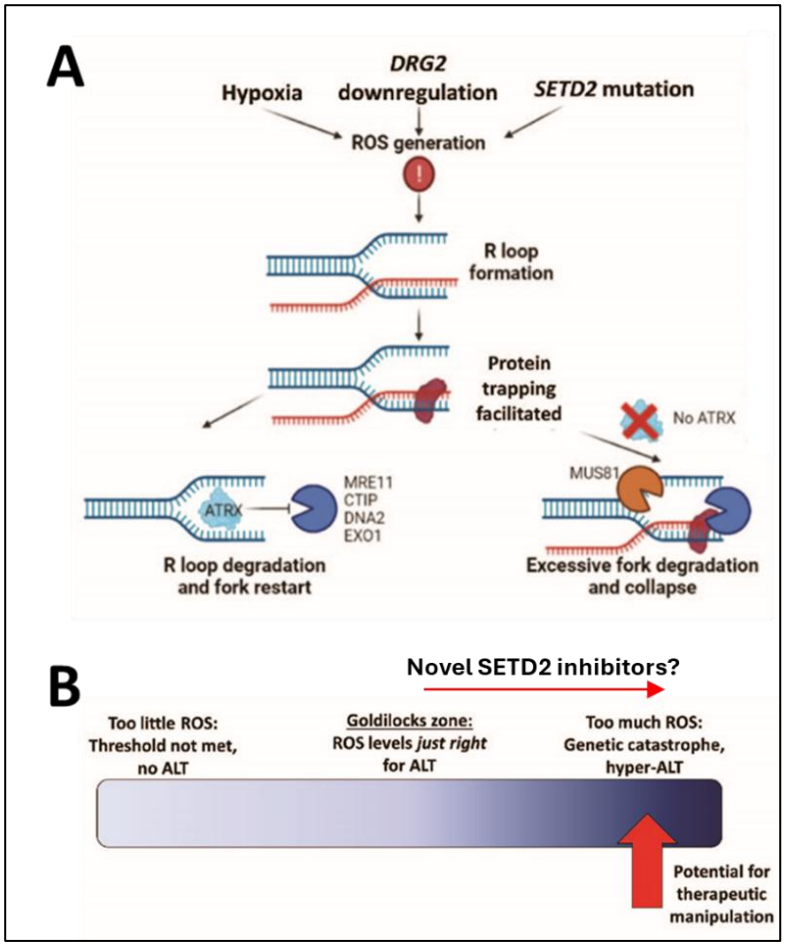
31. Exploring epigenetic changes in paediatric high-grade gliomas with SETD2/H3F3A mutations – Anna Rose

Primary Supervisor: Anna Rose
Additional Supervisors: Ester Hammond / Marketa Tomkova
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract of the project

Telomere maintenance is an essential cancer hallmark, allowing malignant cells to divide without limit. One major telomere maintenance mechanism is called Alternative Lengthening of Telomeres (ALT). The ALT-pathway is particularly prevalent in cancers affecting children and young people – such as aggressive brain cancers (high-grade glioma, HGG) and osteosarcoma [1]. The central genetic event underpinning ALT-pathway activation is loss of ATRX [2]. In addition to ATRX loss, ALT-pathway activation requires another factor.



Our recent work demonstrated that this second factor is excessive accumulation of reactive oxygen species (ROS) in the tumour microenvironment, which could be due to hypoxia, concurrent gene mutation and/or redox gene dyrsregulation [3]. Elevated ROS levels lead to trapping of DNA-interacting proteins, which subsequently causes replication fork stalling and ALT-pathway activation [3,4]. ATRX protein is essential for fork re-start and so, in the absence of ATRX, there is aberrant downstream processing of stalled forks. This aberrant processing produces DNA double-strand breaks, the genetic substrate for ALT-telomere elongation (**Figure A**).

We recently identified that concurrent mutation of ATRX and SETD2 often occurred in paediatric HGG [3]. SETD2 is a histone methytransferase, responsible for H3K36 trimethylation. Loss of SETD2 leads to loss of this essential epigenetic mark. It has also been postulated that this pattern is mimicked by the H3 p.G34R mutation, which is also very common in paediatric HGG. Curiously, loss of SETD2 appears to cause elevated oxidative stress, through dysregulation of redox genes. In this project, we will explore the epigenetic changes in SETD2/H3F3A mutant HGG, with the aim of understanding the pattern of gene dysregulation and exploring why this leads to elevated oxidative stress.

Research objectives and proposed outcomes

Novel SETD2 inhibitors - EZM0414 has been recently fast-tracked by the FDA as a novel first in class inhibitor of SETD2 for use in some adult cancer types, such as lymphoma. We would like to test this agent in various cell

models, to assess whether SETD2 inhibition (in ALT-positive tumours which have *not* lost this gene) can potentiate ALT pathway activity. We would also like to explore the downstream effects of SETD2 inhibition, including ROS generation, level of ALT pathway activity (c-circles, APBs, telomere length) and cell viability (clonogenic assay). We think that further elevation of ROS will lead to hyper-activity of the ALT pathway, which leads to genetic instability and cell death (**Figure B**). This aspect of the work could be performed in a 6-month rotation for stream 3 applicants.

Epigenetic dysregulation in SETD2 mutant and H3G34R mutant: SETD2 is a H3K36 methyltransferase. The common H3F3A mutation, p.G34R, appears to cause steric inhibition of this same histone mark. Our recent data suggests that SETD2 loss causes elevated ROS. This will be interrogated further, through various techniques such as methylation array, ChIP-seq (to identify which genomic regions are perturbed in the mutants) and RNA-seq to epigenetic changes with downstream expression profile changes. This work will be performed in various cells line which we have engineered in the lab, including ATRX, SETD2 and H3F3A mutant high grade glioma cell lines.

Functional consequences of redox gene dysregulation and elevated ROS: a key question is by what mechanism does elevated ROS lead to ALT pathway activity. This aspect would be explored in the latter stages of the project, and might involve assessing direct base damage (8oxoG), non-canonical DNA structures (e.g. R loops, G-quadruplexes) and DNA-protein complexes. This would involve a variety of techniques, including blotting, immunoprecipitation, immunofluorescent imaging, HPLC-MS and, potentially, structural biology techniques.

Translational potential of the project

Development of novel therapeutics for ATRX-deficient cancers is an urgent area of clinical unmet need. The outcomes for ALT-cancers is very poor, with little progress made in survival in over 50 years. The work in this project is hypothesis-driven, pre-clinical data, but will be critical in informing future translational work. The insights into gene dysregulation, telomere dysfunction and genome stability will clarify the pathways involved in ALT-cancer biology, which is the first critical step in developing targeted therapies. Testing of newly-licensed SETD2 inhibitors might allow repurposing of these agents, which would have immediate clinical translational benefits.

Training opportunities

Dr. Rose and Prof. Hammond have worked together collaboratively for the past 4 years. They have a strong track record for supervising DPhil, MSc and BSc students. For this project, we have also established a new collaboration of Dr Tomkova, allowing cross-disciplinary collaboration and deeper exploration of the epigenetic alterations in these cancers, as well as capitalising on her expertise in computational biology. This project offers the opportunity to join a well-funded, collaborative and interdisciplinary team. The student will be based in the Rose group (Department of Paediatrics, located within the WIMM), with strong links and support from the Hammond and Tomkova groups. The student will have the opportunity to learn a wide range of molecular and cell biology techniques including tissue culture, protein analysis, gene expression analysis, various telomere assays, immunoflouresnce microscopy and epigenomic techniques, such as methylation analysis and ChIP-seq. The data analysis of these latter aspects will be strongly supported by Dr Tomkova. It will also potentially involve working closely with new international collaborators to develop new techniques for studying telomeric oxidative damage.

References

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32. Integrative genomic approaches to optimize T Cell therapies for cancer – Sumana Sharma

Primary Supervisor: Sumana Sharma
Additional Supervisors: Guilia Orlando
Eligibility: Track 2 applicants are eligible to apply for this project

Abstract of the project
Immunotherapy, which harnesses the immune system to treat disease, is transforming cancer care. Adoptive cell therapy (ACT), where T cells are engineered outside the body and reintroduced into patients, has been successful in blood cancers but remains largely ineffective for solid tumors.
Alongside immune cell-extrinsic factors such as the suppressive effects of the tumour microenvironment, a major barrier to successful ACT is the 'quality' of the T cells used for therapy, which contributes to inefficient persistence, exhausted phenotypes, and poor infiltration of tumours. Recent studies have highlighted distinct T cell differentiation trajectories and functional states across tumor microenvironments, underscoring the necessity to understand precisely what occurs to T cells within each cancer type to optimize their functionality in a context-dependent manner. This proposal aims to understand and manipulate the gene networks that control T cell function within different cancer microenvironments, enabling us to engineer superior T cells tailored for different cancer types and environments.

Specifically, we aim to:

1. Identify and characterize the key differences in cancer-specific T cell states across various tumor types, using integrated multi-omics datasets (single-cell RNA-seq, ATAC-seq, and bulk RNA-seq).
2. Utilize network-based predictive modeling informed by multi-omics data to predict specific transcription factors and signaling network alterations that define optimal T cell functionality for each cancer type. The model will systematically identify transcription factors whose modulation will retain beneficial traits (e.g., robust tumor infiltration) while eliminating detrimental ones (e.g., terminal exhaustion), thereby optiming T cells for each contexts.
3. Systematically screen through predicted signalling and transcription factor candidates inferred from the model using CRISPR-based arrayed and pooled screening approaches, alongside barcoded cDNA overexpression libraries. Engineered T cells will be rigorously evaluated using functional assays measuring cytotoxicity, proliferation, cytokine production, and infiltration into organoid-based cancer models through advanced imaging approaches. We will utiltise the existing colorectal, melanoma, and AML cancer models and develop novel cancer-immune interaction models.

This project will yield a unified epigenomic atlas of context-specific $CD8^+$ T-cell states across representative solid and liquid tumours, an open-source predictive network model that ranks transcription factors and signalling nodes capable of enhancing desirable traits, a prioritised shortlist of experimentally validated gene targets, and proof-of-concept CAR- or TCR-engineered T cells reprogrammed with top context-specific edits that display superior persistence and anti-tumour activity. All barcoded CRISPR libraries, over-expression constructs and protocols will be released to the community, providing a translation-ready toolkit.

This is an intradisciplinary project expertise from genetics, network-biology, immunology, cancer biology, and advanced imaging. The insight from the project will contributes to our understanding of T cell biology in cancer and paves the way for customised immunotherapies. It will also foster collaborations with teams focused on reprogramming cells through transcriptional regulation. With this, it will address a major unmet need in cancer

therapy: the lack of effective immunotherapies for solid tumors. By uncovering and targeting the molecular networks that govern T cell function that is unique to the solid tumor setting, we aim to design T cells that are more persistent, less prone to exhaustion, and better able to infiltrate tumors. These findings could significantly improve the precision and effectiveness of T cell-based therapies, moving toward more personalized and effective treatments for cancer patients.

Training opportunities

1. Single-cell and bulk omics data generation and bioinformatic analysis mainly on network- based approaches.
2. Functional genomic screening using CRISPR-Cas
3. Culturing and editing primary T cells
4. In vitro assays, Flow cytometry, Incucyte-based killing assays.
5. Co-culture experiments with cancer organoids and imaging.

Ideal student background: This is a fully integrated project spanning wet-lab and computational work in cancer immunology. While applicants do not need prior computational expertise, they must be eager to engage with both experimental and data- analysis components.

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33. Structure-based targeting of the BMP antagonist GREMLIN-1 to prevent colorectal cancer – Christian Siebold

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

Abstract of the project
Disruption in the Bone Morphogenetic Protein (BMP) pathway is causally implicated in the initiation of intestinal polyposis syndromes. Patients identified with these high risk conditions have no therapeutic options are face a lifetime of intrusive surveillance or prophylactic colectomy. Existing preclinical evidence demonstrates that manipulation of the BMP pathway by inhibition of the secreted antagonist GREMLIN1 prevents polyposis progression and improves survival. Structural modelling of the GREM1 interaction with BMP ligands shows a potential targetable small molecule binding site in the GREM1-BMP interface that undergoes a structural transition when bound to BMP ligand. The student will undertake protein crystalisation and fragment screening, working towards the identification of a novel small molecule inhibitor, test compound affinity and then interrogate efficacy of potential agents in organoid and mouse models.

Background. The Bone Morphogenetic Protein (BMP) pathway is a critical morphogen signalling pathway and a key mediator of intestinal homeostasis. BMP signalling acts cross-compartmentally, with stromal cell ligand expression acting pleiotrophically in a paracrine and autocrine fashion to induce epithelial cell differentiation at the luminal surface of the crypt and regulate fibroblast cell functional heterogeneity. In order to avoid inappropriate differentiation of crypt basal stem cells, BMP ligands are excluded from the crypt basal stem cell niche by the restricted expression of secreted ligand sequestering BMP antagonists such as GREMLIN1 (GREM1) from sub-cryptal myofibroblast cell populations (yellow dots - Figure 1). As a strictly regulated mediator of intestinal homeostasis, disruption in the BMP pathway is causally implicated in colorectal cancer (CRC) initiation and progression. Disruption of BMP signalling gradients through germline mutations in either the BMP receptor (BMPR1A) or signal transduction (SMAD4) are responsible for Juvenile Polyposis Syndrome (JPS) [1]. In line with this, aberrant epithelial expression of GREMLIN1 induces aberrant stem cell behaviour in Hereditary Mixed Polyposis Syndrome (HMPS) [2]. Furthermore, inherited variation in the BMP pathway is arguably the major influence on CRC risk in the general UK population, with at least 8 single nucleotide polymorphisms (SNPs) close to GREM1 and BMP ligands being independently associated with risk of CRC in white northern Europeans, and probably in other ethnic groups [3]. Consequently, the BMP pathway is an attractive target for therapeutic manipulation in a cancer prevention context, to generate new drugs for patients with genetically identified germline polyposis syndromes who currently have few therapeutic options to prevent future cancer progression.

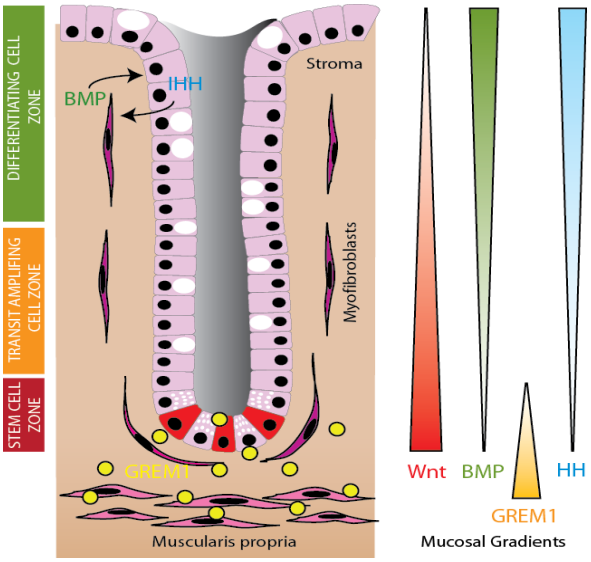

Fig. 1. Signalling regulation of intestinal homeostasis

Preliminary data. This project looks to target the BMP pathway, through its pleiotropic, secreted antagonist GREM1. Work from ourselves and others has shown:

1. Aberrant epithelial expression of GREM1 is responsible for ectopic stem cell behaviour and polyp initiation in Hereditary Mixed Polyposis Syndrome [4]
2. Using mouse models of HMPS polyposis, we can completely abrogate the HMPS polyposis phenotype through the use of a GREM1 therapeutic sequestering antibody [2]. However this antibody is not suitable for long term use in a cancer prevention setting, resulting in an urgent need for a functionally equivalent small molecule inhibitor.
3. Manipulating BMP signalling genetically through expression of BMP4 ligand or antibody inhibition of GREM1 reduces cancer stem cell activity and profoundly slows progression of a mouse model of Familial Adenomatous Polyposis, doubling animal lifespan (unpublished).
4. Structural modelling of the GREM1 interaction with BMP ligands shows a potential targetable small molecule binding site in the GREM1-BMP interface that undergoes a structural transition when bound to BMP ligand.
5. We can produce milligram quantities of monodisperse, bioactive human GREM1 protein that can be used for structural and functional studies. We have also crystallisaed apo GREM1 and determined its structure to sub 3 Å resolution.

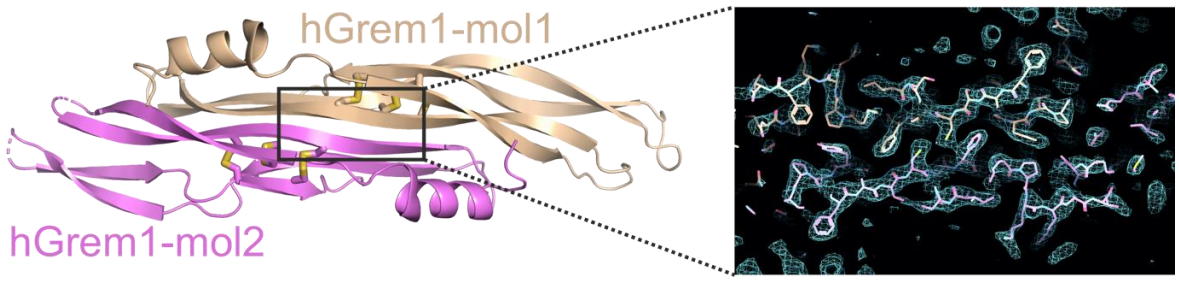

Fig.2: Crystal structure of our GREM1 dimer with experimental map shown in inset (unpublished). The purified protein sample used for crystallisation is able to stimulate organoid growth and efficiently inhibits BMP signalling in cells. This crystallization condition will form the basis of our planned fragment screen to find inhibitors of GREM1 signalling.

Research objectives and proposed outcomes

1. **Protein crystalisation and fragment screening.** Based on our promising preliminary structural results, the crystallisation procedures will be optimised to produce a large number of GREM1 crystals for structure-based fragment screening using our high throughput nanolitre crystallisation facility at the Division of Structural biology (STRUBI). Siebold is the lead PI of the Oxford Beamtime Allocation group that provides ready access to state-of-the-art X-ray crystallography beamlines at the UK synchrotron Diamond Light Source (DLS). In collaboration with the fragment screening team at DLS beamline I04-1, freezing, soaking conditions and data collection strategies of GREM1 crystals will be optimised. Objective is to collect 500-1000 different datasets allowing screening of >20,000 compounds.

2. **Affinity testing**. Once small molecule binders have been identified, binding affinities will be determined using surface plasmon resonance and/or biolayer interferometry. The Siebold group has access to and vast experience in the use of these methods. The most promising compounds will be selected for follow-up functional experiments in cellular and organoid models.

3. **Functional testing of identified agents.** Identified compounds will be tested for Grem1 inhibition efficacy using intestinal organoid systems as the growth of these *in vitro* cultures is dependent on the activity of media supplemented BMP antagonists like Grem1. Outcome measures will include organoid growth and budding. In parallel, compounds will be tested in cellular response assays based on a luciferase reporter. Efficacious agents will be further tested in a mouse model of HMPS (*Vil1-Grem1*), to look for abrogation of the polyposis phenotype

Translational potential of the project

Patients with genetically identified germline polyposis syndromes have few therapeutic options. Current management involves regular (often annual) intrusive surveillance of polyp burden through colonoscopy, with prophylactic colectomy recommended when the polyp burden becomes endoscopically unmanageable. Even after colectomy, patients can be affected by disease in other parts of the gastrointestinal tract such as the stomach and duodenum. There have been few advances in chemoprohylaxis with aspirin and other NSAID's the only drugs that have generated any measureable impact. Although effective in manipulating BMP/GREM1 signalling and reducing polyp burden in mouse models, the GREM1 sequestering antibody we have previously used has no therapeutic window for long term cancer prevention in polyposis syndromes. There is increasing interest and focus in understanding precancer biology to deliver prevention opportunities and this project combines solid preclinical data background with the tools and capacity for novel small molecule drug design. It is anticipated that a successful project would lead to IP protection and consideration of clinical trial design.

Training opportunities.

This project sits at the intersection of structural biology, drug design and biological application, and the student will benefit from exposure to all these aspects. They will learn structural techniques such as protein purification and crystallisation, high throughput fragment screening for target identification through medicinal chemistry drug optimisation, and onwards to application and testing in organoid model systems and mouse models.

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34. Study the role of pseudouridine synthases in cancer with a zebrafish model – Chunxiao Song

Primary Supervisor: Chunxiao Song
Additional Supervisors: Richard White
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract of the project
In recent years, there have been major breakthroughs in discovering RNA-modification-mediated biological regulation, leading to the emerging field of epitranscriptomics. Pseudouridine (Ψ) is the most abundant modification in cellular RNA which is introduced post-transcriptionally by pseudouridine synthases (PUS). Despite its prevalence, the biological functions of Ψ and PUS enzymes remain poorly understood. Recently, we developed a novel sequencing method, called BACS, for quantitative detection of Ψ at single-base resolution. BACS allows comprehensive identification of PUS targets, which provides a great opportunity to study their biological functions. To identify their biological importance in cancer, this project aims to take full advantage of the high-throughput potential of the zebrafish model to investigate the tissue-specific roles of PUS enzymes in vivo, aiming to uncover how their enzymatic activities contribute to tumorigenesis.

Research objectives and proposed outcome
Pseudouridine (Ψ) is the first identified and the most prevalent post-transcriptional RNA modification, particularly abundant in non-coding RNAs, including ribosomal rRNA (rRNA) and transfer RNA (tRNA)[1]. Ψ is installed by pseudouridine synthases (PUSs) and thirteen putative PUS enzymes have been annotated in the human genome[2]. Ψ plays important roles in translation, splicing, and RNA stability, and dysregulation of PUS enzymes has been implicated in various diseases including cancer[3]. However, the biological functions of pseudouridylation and PUS enzymes remain poorly understood, largely due to the lack of highly sensitive and accurate detection methods and limited knowledge linking specific PUS enzymes to their targets.

Recently, we developed a new sequencing method, 2-bromoacrylamide-assisted cyclization sequencing (BACS), for direct, quantitative, and base-resolution sequencing of Ψ[4]. BACS serves as a valuable tool for studying Ψ modifications, not only provides a comprehensive and accurate picture of the Ψ landscape across the human transcriptome, but also enables us to investigate the biological functions of Ψ, the enzymatic properties of PUS enzymes, and the relationship between PUS enzymes and cancer. For example, we have applied it to studying the targets of three key PUS enzymes (TRUB1, PUS7, and PUS1) in HeLa cells and revealed these three PUS enzymes had broader targets than previously thought[4]. This represents a great opportunity to study how dysregulated pseudouridylation and PUS enzymes contributes to tumorigenesis.

A major challenge in understanding the role of PUS enzymes in cancer is to identify their biological importance and understand how their biochemical activities contribute to specific phenotypes in vivo. Many of PUS enzymes are ubiquitously expressed yet lead to tissue specific effects. Understanding this requires a model system that is amenable to rapid genetic manipulation and allows us to interrogate tissue specific phenotypes. While mice are a common model, they are time- and resource-intensive and it is difficult to test multiple genes in a scalable manner. For these reasons, this project will utilize the unique capabilities of the zebrafish (Danio rerio) to study this problem. The zebrafish is a small, transparent vertebrate that is highly amenable to imaging and rapid genetic manipulation[5]. The pseudouridylation and PUS enzymes are conserved in zebrafish, which is also highly amenable to modelling of many different types of cancers using genetic approaches[6]. We will study tissue specificity of PUS enzymes within the whole zebrafish, combined with BACS and other molecular techniques to study the pseudouridylation.

Aim 1. CRISPR screening in zebrafish embryo provides a rapid and scalable and genetic method testing gene function in a single generation. We will first establish a CRISPR-based knockout screen targeting the 13

pseudouridine synthase (PUS1, PUSL1, PUS3, PUS7, PUS7L, PUS10, TRUB1, TRUB2, RPUSD1–4, and DKC1) genes in zebrafish. This will be done using either mosaic Cas9-sgRNA complexes (to induce mutations) or Cas13-sgRNA complexes (to induce gene knockdown). We will use an automated injection robot into 1-cell zebrafish embryos with sgRNAs targeting each of the PUS enzymes. The fish will then be monitored using whole-embryo imaging to look for specific defects in developmental patterning (i.e. heart, skin, intestine, brain development). For the most promising mutants, we will perform whole-embryo single-cell RNA-seq using the high-throughput sci-Plex method, which allows for cost-effective sequencing of all cells in the animal[7]. This goal is to determine which cells are most affected by the candidate PUS enzyme, and what molecular pathways are altered within those cells.

Aim 2. We will elucidate the molecular mechanisms driving the most prominent tumorigenic phenotypes from Aim 1. Using a combination of biochemistry, molecular biology, genetics, and sequencing techniques such as BACS and ribosome profiling, we aim to directly link the enzymatic activity of PUS enzymes to specific cancer-related phenotypes, identifying underlying molecular mechanisms.

Translational potential of the project

This project will address critical gaps in understanding how pseudouridylation and PUS enzymes influence tumorigenesis and identify specific PUS enzymes that are critical in driving tumorigenesis, including tissue-specific cancer initiation. These insights may reveal epitranscriptomic vulnerabilities that can be therapeutically targeted, informing the development of novel cancer treatments.

Training opportunities

The student will join a dynamic research environment at Ludwig Cancer Research, and benefit from the combined expertise of the Song group (chemical biology, epigenetic and epitranscriptomic sequencing) and the White group (zebrafish genetics and cancer). Some specific skills that the student will gain:

- Biochemical assays and molecular biology techniques
- Epitranscriptomic sequencing, including BACS
- Zebrafish genetic techniques including transgenes and CRISPR modifications
- High resolution live cell imaging
- Single-cell transcriptomics

Ideal student background: Students from with a Biochemistry or Genetics or a related science background are welcome to apply. A strong background in molecular biology, genetics, and cell biology is desirable.

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35. Combining polylipidoid and microneedles for safe and effective intradermal cancer vaccines –Molly Stevens

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

Abstract of the 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.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 formulations.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, leading 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 biocompatibility.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 polylipidoid-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.
3. **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 polylipidoid 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 Licence for conducting animal research.

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

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

References

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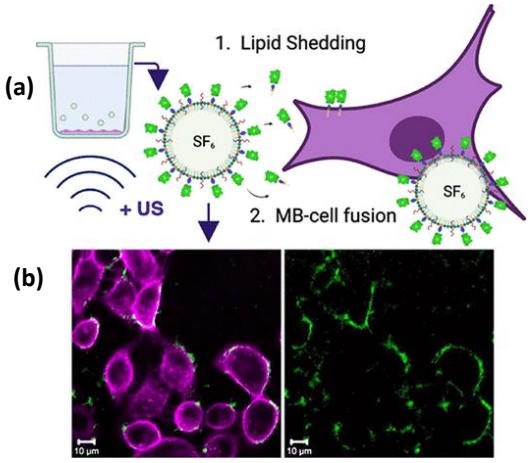
36. Ultrasound triggered cancer cell-tagging for radioimmunotherapy in glioblastoma multiforme – Eleanor Stride

Primary Supervisor: Eleanor Stride
Additional Supervisors: Edward O'Neill
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract of the project

Recent studies have unveiled important functions of plasma membrane lipid and cell surface signaling molecule dynamics in regulating cancer cell immunogenicity, and, hence, modulation of these membrane lipids can be exploited to harness T cell activity[1]. Delivering therapeutic material to the membranes of specific cells in the body, however, poses a major challenge. For example, modifying T cells to display chimeric antigen receptors (CARs) has proven to be a highly successful strategy for certain types of cancer, but this process is technically challenging and prohibitively expensive[2]. We have recently demonstrated that protein-decorated gas microbubbles (MBs) that can be activated using ultrasound (US) can successfuly "tag" A459 lung carcinoma cell membranes with a specific proteins (e.g. GFP, transferrin)[3]. The aim of this project will be to build on this proof-of-concept study and undertake a more detailed analysis of the mechanism(s) of tagging, to develop an optimised microbubble formulation and ultrasound exposure protocol, to use this approach to insert a "foreign" protein into the plasma membrane of glioblastoma multiforme (GBM) cells and to investigate whether the transplanted protein functions as a therapeutic target for antibody-based therapeutics (such as radioimmunotherapy).



*(**a**) Cells are incubated with microbubbles (MB) and exposed to US resulting in lipid-shedding and "membrane tagging" of recipient cells with transferred protein. (**b**) Confocal microscopy images of A549 lung carcinoma cells stained with Cell Mask Deep Red (magenta) and His-GFP lipids (green) transferred from MBs after 60s of US exposure.*

Research Objectives and proposed outcomes

(**1**) The first objective of the project will be to develop the existing microbubble formulation to load a targetable protein into GBM cell membranes and to characterise the loading efficiency and protein functionality. We have established protocols for microbubble formulation and characterisation, including their conjugation to different types of protein. We will test a panel of protein candidates aiming for antigens not naturally expressed in GBM (e.g. CEA). (**2**) The microbubble formulation will be tested for its ability to transfer protein to GBM cell membranes following ultrasound exposure and the resultant activity of those cells. This stage of the work may include modifying the formulation to enhance cellular targeting, e.g. through the addition of a GBM-targeting protein. (**3**) The abundance of transferred protein in recipient cells will be evaluated using confocal microscopy and flow cytometry. (**4**) Antibody binding to the transferred protein will be tested in saturation and competitive binding assays. (**5**) The cytotoxity of antibodies directed against the transferred protein with or without a payload (e.g. therapeutic radioisotope) will be tested *in vitro* and in *in vivo* murine xenograft models of GBM. A stretch goal will be to study the effect of this approach in a syngeneic murine model of GBM (GL261).

Translational potential of the project

GBM is a cancer of unmet clinical need with poor survival and limited effective treatment options. Surgical excision is a mainstay of treatment but local recurrence due to infiltration by GBM cells into the nearby cerebral parnenchyma is common. The surgical cavity presents an opportunity to instil a local treatment that could tag

residual GBM cells with a unique protein target for subsequent antibody-based therapy. It is anticipated that the proposed therapy could be delivered using existing clinical devices. Microbubbles have been in clinical use as contrast agents for several decades and Prof. Stride's team have developed methods for producing drug-loaded microbubbles that have recently been approved for clinical trials. The translational potential of the project

Training opportunities
This is an interdisciplinary project between the Departments of Oncology, the Nuffield Department of Surgical Sciences, Engineering Science and the Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Science. Successful candidates will benefit from training in cell culture, flow cytometry, immunology, microbubble formulation, microscopy, therapeutic ultrasound, antibody therapeutics and statistical analysis. They will join a multidiscplinary research team with a strong track record of collaborative research and state of the art laboratory facilities.

Ideal student background: Candidates should have either some basic experience and understanding of cell culture and cancer biology; or experience with formulation and characterisation of drug delivery systems.

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37. Investigating the Immune Biology of CAR T-Cell Neurotoxicity (ICANS) in Lymphoma Patients - Bo Sun

Primary Supervisor: Bo Sun
Additional Supervisors: Maria Isabel Leite
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract of the project
Chimeric Antigen Receptor (CAR) T-cell therapy has revolutionised treatment for relapsed or refractory B-cell lymphomas, achieving remission rates up to ~80% in patients who failed conventional therapy[1]. However, this breakthrough can come at a cost: up to one-third of patients experience serious immune-related side effects, notably cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS)[2]. ICANS is a therapy-induced neurologic syndrome ranging from mild confusion to life-threatening seizures, brain swelling and coma. Emerging evidence on long-term outcomes now suggest as much as a third of patients experience persisting neurological disability[4]. The precise immune mechanisms driving ICANS remain poorly understood[3], making it difficult to predict or prevent. This project will investigate the immune biology of CAR T-cell mediated ICANS in adult lymphoma patients treated with CD19 or BCMA-targeted CAR T therapies. We will leverage proteomics, single-cell RNA sequencing, and *in vitro* models on patient blood, serum, cerebrospinal fluid (CSF), and CAR T-cell products. Our aim is to identify early biomarkers and unravel the cellular and molecular pathways underlying ICANS. Ultimately, this research seeks to improve our ability to predict ICANS risk and inform strategies to mitigate neurotoxicity, enhancing both the safety and efficacy of CAR T-cell therapies.

Research objectives and proposed outcomes

Objective 1: Identify predictive biomarkers of ICANS:
We will analyse patient samples (blood, CSF, etc.) for candidate proteins/genes that distinguish patients who develop ICANS from those who do not. Unbiased proteomic and single-cell transcriptomic profiling will aim to pinpoint cell subsets or gene expression changes associated with neurotoxicity. *Proposed outcome:* a panel of candidate biomarkers (e.g. cytokines, immune cell subsets, or gene signatures) that can accurately forecast ICANS risk before or early during therapy. This would have high academic and clinical value by revealing key mediators of neurotoxicity and could lay the groundwork for a predictive clinical test.

Objective 2: Uncover immune mechanisms of ICANS:
Using an unbiased single-cell approach, we aim to characterise how CAR T-cells and the patient's immune system interact to cause neurological side effects. We will examine CAR T-cell products and serial patient samples to see how immune cell phenotypes evolve in patients with ICANS. This includes determining if specific CAR T-cell subpopulations (for instance, overly activated "exhausted" T cells or CAR T cells with inflammatory profiles) or host immune cells (such as myeloid cells crossing into CSF) are driving the syndrome. *Proposed outcome:* a mechanistic model of ICANS identifying which cells, molecules, and pathways in the blood/CSF are responsible for blood-brain barrier disruption and neurotoxicity. Academically, this advances fundamental understanding of neuro-immune interactions in the context of cancer immunotherapy, a currently underexplored area.

Objective 3: Functional validation of candidates ICANS meditors in vitro
Building on candidate biomarkers or molecular pathways identified through single-cell and proteomic profiling (Objectives 1 and 2), we will conduct *in vitro* studies to investigate their functional role in ICANS biology. Using co-culture models of CAR T cells and relevant immune/lymphoblastoid cells, we will test how specific cytokines, transcriptional programs, or immune cell subsets influence neurotoxicity-associated phenotypes. *Proposed outcome:* Experimental validation of whether the biomarkers identified in patient samples are **drivers** or merely **correlates** of ICANS.

Translational potential of the project
The project is inherently translational and is supported by a strong collaboration between the university's research labs and the hospital's Haematology service. As part of this project, the DPhil candidate will work closely with clinician collaborators who will identify and recruit adult lymphoma patients undergoing CAR T therapy. ICANS is a pressing clinical problem in cancer care and represents a significant health and economic burden to current and future cellular therapy services. Severe neurotoxicity can necessitate intensive care support and may be fatal in rare cases, limiting the broader use of CAR T therapies. By identifying robust biomarkers and clarifying ICANS mechanisms, this project has direct implications for improving patient management.

Training opportunities
This DPhil project provides broad, interdisciplinary training spanning immunology, genomics, and clinical cancer research. The student will gain hands-on experience with single-cell RNA sequencing, including sample preparation from blood, CSF, and CAR T-cell products. They will receive bioinformatics training in data processing, integration of multi-omics datasets, building strong computational skills.

The student will learn immune profiling techniques, including molecular cloning, cytokine profiling, and functional T-cell assays, enabling them to link immune phenotypes with biological activity.

Given the project's clinical setting, the student will have the opportunity to develop translational research skills, including ethical handling of patient material, interpretation of clinical data, and collaboration with multidisciplinary clinical care teams.

Ideal student background: Ideally candidates should hold a bachelor's degree or master's degree in Medicine, Medical Sciences, Chemistry, Biochemistry, Mathematics, Engineering or Computer Science, with at least upper second-class honors (if applicable). An MSc in relevant areas may be advantageous or relevant experience e.g. experience in industry, research employment or relevant publications of peer-reviewed research articles

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

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

Abstract of the project
Identifying and characterising genetic risk factors is essential for understanding cancer susceptibility and informing prevention strategies. Although genetic predisposition cannot be modified, it offers unique insight into the biological mechanisms underlying carcinogenesis and holds promise for preventive approaches, such as chemoprevention, analogous to cardiovascular risk management with statins and antihypertensives.

While large-scale studies have uncovered many inherited cancer risk factors over the past decade, a substantial proportion of risk remains unexplained. This is partly due to the limited resolution of standard techniques, which often miss certain classes of variation, including short tandem repeats (STRs). STRs are highly polymorphic repeat elements that can affect gene expression, particularly in regulatory regions, but have remained understudied due to technical challenges in genotyping from short-read data.

This project leverages whole-genome sequencing (WGS) data from large cohorts (e.g. 100,000 Genomes Project, UK Biobank) to systematically investigate the role of inherited STR variation in colorectal and other cancers. It will also explore the role of acquired STR mutations, especially in mismatch repair–deficient tumours where STR instability is common. The project includes a flexible mix of computational genomics and laboratory-based validation, depending on emerging results and student preference.

By systematically analysing STRs, this project addresses a major gap in our understanding of inherited cancer risk, which is often referred to as the "missing heritability." STRs represent a largely untapped class of genetic variation with the potential to act as biomarkers for both risk prediction and early detection. Emerging evidence suggests that their influence on gene regulation, genome stability, and tumour evolution could make them valuable tools for improving risk stratification models. This work could ultimately transform how we identify high-risk individuals, opening new avenues for targeted surveillance, chemoprevention, and precision oncology. The project combines novelty with translational promise, extending the supervisors' proven success in cancer gene discovery into a new and understudied genomic frontier.

Research objectives and proposed outcomes

1. Catalogue variable microsatellite loci across the human genome using existing reference datasets.
2. Develop robust methods to determine allele lengths from short- and long-read sequencing of germline DNA.
3. Integrate public and in-house datasets to assess associations between microsatellite variation and gene expression in normal and tumour tissues.
4. Prioritise microsatellites that impact gene expression or function, including those in coding regions or regulatory elements.
5. Test selected microsatellites for inherited differences in large colorectal cancer case-control cohorts to identify novel risk loci.
6. Depending on results and student interest, extend the analysis to other repeat types or undertake laboratory studies on selected target genes.
7. If time permits, investigate somatic microsatellite mutations in cancers, focusing on mismatch repair–deficient tumours with elevated microsatellite instability.

Translational potential of the project

This project aims to clarify the contribution of short tandem repeats (STRs) to inherited cancer susceptibility. It may also address STRs as somatic driver mutations in colorectal and other cancers, especially with regards to genetically unstable cancers and driver mutations linked to STR instability across cancer types. By improving our understanding of this overlooked class of variation, the findings could support more accurate risk prediction models and, in the long term, inform the development of targeted prevention and treatment strategies.

Training opportunities

The student will be trained in statistical genetics, bioinformatics, and genetic epidemiology, including the use of tools such as R, Unix, and command-line software. They will gain experience working with large-scale genomic datasets within secure research environments (e.g. UK Biobank, Genomics England). Optional training in relevant wet-lab techniques will also be available.

Ideal student background: Clinical background with interest or experience in cancer genetics. Exposure to computational genomics or willingness to develop bioinformatic skills would be beneficial.

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39. Beyond tumour control: Investigating brain aging after checkpoint immunotherapy for melanoma – Anya Topiwala

Primary Supervisor: Anya Topiwala
Additional Supervisors: Benjamin Fairfax
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract of the project
Checkpoint inhibitors have revolutionised the treatment of several cancers, particularly melanoma, by enhancing T cell-mediated immune responses against tumour cells through blockade of pathways such as PD-1 (programmed cell death-1). Whilst these therapies have led to significant improvements in cancer remission rates, especially in the metastatic setting, their long-term impact - particularly in younger patients and in the adjuvant context - remains less clear, with limited survival benefit in some cases. Growing attention is being paid to immune-related adverse events (irAEs), which result from off-target T cell activity affecting healthy tissues. Among these, neurological irAEs occur acutely in approximately 1-5% of patients, [Farina et al., 2024] yet the potential chronic effects of checkpoint inhibitors on the brain remain unexplored. There is a strong biological rationale to suspect checkpoint inhibitors could contribute to accelerated brain aging, through mechanisms including chronic inflammation, vascular injury, autoimmune responses targeting neural tissues, and disruption of the blood-brain barrier. [Lou et al., 2024]

Magnetic resonance imaging (MRI) offers a non-invasive, quantitative, and sensitive method for tracking brain changes longitudinally - often years before clinical symptoms emerge. This project will leverage newly available real-world NHS electronic health record (EHR) data and longitudinal brain imaging data available through the Thames Valley and Surrey secure data environment, encompassing data from as early as 2013. Cancer treatment provides is a unique opportunity to study brain aging, as patients frequently undergo repeated imaging (every 3-6 months) over extended periods. Given that randomised controlled trials to detect long-term neurotoxic effects of checkpoint inhibitors would be neither practical nor ethical, this study will apply causal inference methods, specifically an emulated target trial. The project will compare patients with melanoma treated with immunotherapy to matched historical controls (pre-2018) who did not receive checkpoint inhibitors. Longitudinal brain imaging will be analysed using validated automated pipelines. Machine learning models trained on healthy populations (e.g. UK Biobank) will be used to estimate brain age, allowing assessment of whether immunotherapy recipients show an increased brain age gap relative to their chronological age. Finally, associations with later psychiatric, cognitive and dementia diagnoses, as captured in the EHR will be explored.

Research objectives and proposed outcomes
This project aims to investigate the potential long-term effects of checkpoint inhibitor therapy on brain health in patients with melanoma, using real-world clinical and imaging data.

Specific objectives:
a) To determine whether treatment with checkpoint inhibitors is associated with accelerated structural brain aging, as measured by longitudinal MRI-derived brain age metrics (e.g. brain age gap between predicted and chronological age).

b) To assess whether checkpoint inhibitor therapy is linked to increased cerebral markers of cerebrovascular disease, such as white matter hyperintensities, microbleeds, or infarcts, detectable through automated neuroimaging analysis.

c) To identify clinical, demographic, or genetic factors associated with differential vulnerability to immunotherapy-related brain aging, including age, sex, comorbidities, treatment regimen, inflammatory markers, and relevant genetic variants (where available).

This project is expected to yield several key outcomes that will advance understanding of the long-term neurological consequences of checkpoint inhibitor therapy:

i. Quantification of Structural Brain Aging in Immunotherapy Recipients
We anticipate demonstrating whether checkpoint inhibitors are associated with accelerated brain aging, as measured by a greater brain age gap using validated MRI-based biomarkers. This will provide the first large-scale, longitudinal evidence linking cancer immunotherapy to potential subclinical neurotoxicity. This novel application of neuroimaging biomarkers in oncology will bridge cancer medicine, neurology, and computational neuroscience, and may inform future survivorship care guidelines.
ii. Detection of Cerebrovascular Changes Associated with Immunotherapy
The study will identify whether checkpoint inhibitors are associated with increased markers of cerebrovascular disease (e.g., white matter hyperintensities, small vessel disease), providing insight into vascular contributions to cognitive decline in cancer survivors. Findings could stimulate new lines of inquiry into the vascular side effects of immunotherapy, with implications for the use of adjuvant immunotherapy in younger populations as well as stroke and dementia risk in cancer patients.
iii. Identification of Risk Factors for Immunotherapy-Related Brain Aging
By linking imaging outcomes with patient-level clinical and genetic data, we aim to uncover predictors of susceptibility to neurotoxicity—such as age, sex, comorbidities, inflammatory profiles, and genetic predispositions. This precision-medicine approach could guide future risk stratification efforts and contribute to biomarker development in the emerging field of cancer-related neurodegeneration. iv.

Methodological advancement
This study represents a significant methodological innovation as it will be the first to perform large-scale brain imaging analyses within the NHS SDE. By leveraging routinely collected clinical imaging data alongside linked electronic health records, the project will establish a scalable and reproducible framework for conducting real world neuroimaging research in oncology and beyond. In addition, this project fosters novel interdisciplinary collaboration between experts in late-life psychiatry, medical oncology, neuroimaging, statistics, and health data science. This cross-departmental approach will integrate clinical insight with advanced computational methods, including machine learning and causal inference, to interrogate complex longitudinal data.

Translational potential of the project
This project addresses a critical knowledge gap in cancer survivorship by exploring the long-term neurological consequences of checkpoint inhibitor therapy. While these immunotherapies have transformed outcomes for patients with metastatic melanoma and other cancers, their potential impact on the brain—particularly in younger patients expected to live for many years post-treatment—remains poorly understood.

By applying advanced brain imaging and real-world data analytics, this study will:

• Provide early evidence of neurotoxic or cerebrovascular effects of checkpoint inhibitors, which may inform long-term monitoring and survivorship care.

• Identify subgroups of patients at higher risk of immunotherapy-related brain aging, supporting the development of personalized risk stratification tools.

• Lay the groundwork for integrating neurocognitive monitoring into routine oncology follow-up, especially in populations exposed to prolonged immunotherapy.

This project expands the focus of immunotherapy research beyond tumour control, addressing underexplored late effects that may significantly impact quality of life, cognitive function, and mental health. Findings could directly influence clinical practice by guiding post-treatment surveillance strategies, patient counselling, and early interventions to mitigate long-term brain health consequences—especially as checkpoint inhibitors are increasingly used in earlier disease stages and younger populations. 4. Summarise the training opportunities

Training Opportunities
This project offers a rich and interdisciplinary training environment, equipping the student with advanced skills across neuroimaging, clinical data science, and translational cancer research. The student will gain hands-on experience with the following techniques and methods:

i. Advanced Neuroimaging Analysis
• Preprocessing and analysis of structural MRI data using established tools (e.g. FSL).
• Application of brain age prediction algorithms using machine learning models trained on healthy populations.
• Quantification of imaging biomarkers of brain aging and cerebrovascular disease (e.g. white matter hyperintensities, atrophy, infarcts).

ii. Real-World Health Data Science
• Working with anonymised patient data within a Secure Data Environment (SDE), including linked NHS electronic health records and imaging archives.

• Data cleaning, integration, and longitudinal structuring of routine clinical datasets.
• Exposure to best practices in data governance, security, and ethical handling of health data.

iii. Causal Inference & Epidemiological Methods
• Designing and implementing an emulated target trial, including cohort definition, confounder control, and sensitivity analyses.
• Statistical modelling of longitudinal brain changes and clinical outcomes using tools such as R.
• Training in contemporary approaches to causal inference from observational data, including propensity score methods and inverse probability weighting.

iv. Interdisciplinary Collaboration and Academic Development

• Regular supervision and mentoring from experts across psychiatry, oncology, neuroimaging, biostatistics, and software engineering.
• Opportunities to contribute to peer-reviewed publications, present at national/international conferences.
• Access to institutional training resources (e.g. methods workshops, data science seminars).

Ideal student background: You will be enthusiastic, motivated with good attention to detail and highly organised. A background in a quantitative/computational field will be helpful. The supervisors are happy to discuss informally with prospective candidates.

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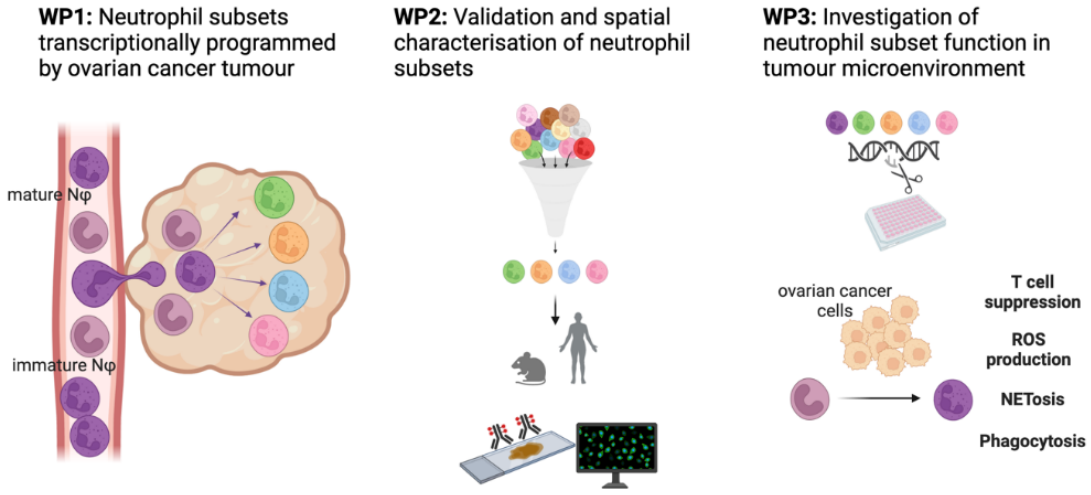
40. Spatiotemporal heterogeneity of neutrophil subsets in ovarian cancer – Irina Udalova

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

Abstract of the project
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.

(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.

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 such as 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 multiplex microscopy and spatial transcriptomics (CosMx Nanostring and Xenium 10X platforms) to define the localisation of myeloid cell subsets within the tumour microenvironment are available.

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

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41. Development of an intra-operative mass-spectrometry tissue classifier system to improve extent of tumour resection in cancer surgery – Claire Vallance

Primary Supervisor: Claire Vallance
Additional Supervisors: Puneet Plaha, Olaf Ansorge
Eligibility: Track 1 and Track 2 applicants are eligible to apply for this project

Abstract of the project
Tumour invasion into surrounding normal tissue is a pathological hallmark observed across a wide range of cancers. In the context of cancer surgery, accurate identification of the tumour margin is critically important. Complete macroscopic excision may be curative in less aggressive cancers or, in more malignant subtypes, improve response to adjuvant therapies such as chemotherapy and radiotherapy. To maximise the chance of complete resection, surgeons often remove an additional margin of surrounding tissue beyond the visible edge of the tumour—at the cost of potential iatrogenic injury to adjacent healthy tissues. Intra-operative histopathological analysis of tissue biopsies can help define the limit of resection margins, but is time-consuming (~30 mins) and resource-intensive. There is increasing interest in rapid diagnostic tools capable of distinguishing tumour from normal tissue in real-time. This project aims to develop a novel intra-operative tissue classifier system that combines ambient ionisation mass spectrometry with machine learning techniques to rapidly differentiate tumours based on their metabolic and lipid profiles. The method will be developed and validated across multiple tumour types, including brain, kidney, and skin cancers.

Research objectives and proposed outcomes
The goal of most cancer resection surgery is complete excision of the tumour, since residual disease often affects prognosis, overall survival and necessitates adjuvant treatment, at significant cost to the patient and healthcare system

[1]. Reliable identification of tumour margins during surgery can be challenging, and frequently relies upon subjective assessment of tissue appearances by the operating surgeon. While histological examination of cytological smears can help differentiate tissues, its application is currently limited by the time (e.g. 30 mins/slide) and resources involved

[2]. As a result, there is growing interest in the use of mass spectrometry (MS) to identify and classify tumours by their metabolic signature. This has been facilitated by advances in ambient ionisation MS (AIMS) technology, which now requires minimal sample processing for analysis

[3]. Systems that translate AIMS to the intra-operative setting have been developed, including the iKnife [4–6] and SpiderMass [3] devices. However, their application in cancer surgery has been limited by their need to burn or vaporise tissue prior to analysis, precluding use in fields such as neuro-oncology surgery where they pose a risk of injury to surrounding delicate neural tissues. This project aims to develop an alternative, non-destructive intra-operative tissue classification system using Atmospheric Solids Analysis Probe Mass Spectrometry (ASAP-MS), coupled with machine-learning-based spectral classification. The overall outcome is a generalisable platform that can aid real-time surgical decision-making across multiple cancer specialties.

Research objectives:

Objective 1: Develop and optimise an ASAP-MS method for the diagnostic classification of brain tumours

- Build a reference library of metabolomic profiles from prospectively collected fresh tumour and margin tissues

- Train and validate a machine learning classifier (e.g. SVM, random forest) using current neuropathological analysis methods as a gold-standard
- Compare diagnostic accuracy of the ASAP-MS method with the current gold-standard method of intra-operative smear cytology and final integrated histomolecular diagnosis

Objective 2: Application of the ASAP-MS tissue classification method to other cancer specialities

- Apply the ASAP-MS tissue classifier method to other cancer specialities where resection margins are critical but also difficult to assess (e.g. renal cell carcinoma, basal cell skin cancer)
- Determine the comparative diagnostic accuracy of the ASAP-MS method across a range of cancer surgery types

Objective 3: Integration of rapid mass-spectrometry into the surgical workflow

- Explore methods to integrate the tissue classifier system into the operative workflow by developing adaptations to existing surgical devices to sample tissues.
- Pilot integration of the method into the surgical workflow and assess impact on extent of tumour resection as well as time- and cost-effectiveness

Translational potential project of the project

The development of an ASAP-MS rapid intra-operative tissue classifier system has the potential to revolutionise various aspects of cancer surgery:

- Diagnostic confirmation of cancer subtypes during an operation would enable surgeons to modify their operative goals based on tumour phenotypes. For example, in neuro-oncology surgery, identification of an IDH-mutant astrocytoma vs an oligodendroglioma may lead to modification of surgical approach due to the different prognostic outcomes for the two tumour types and their responses to adjuvant radiotherapy and chemotherapy.
- Improved delineation of tumour margins would help surgeons to maximise the extent of tumour resection while also minimising the risk of iatrogenic injury from inadvertent resection of healthy adjacent tissues
- Reduced reliance upon manual intra-operative analysis of tissue by a histopathologist would help to minimise operation times and resource use, in the context of significant clinical and financial pressures on cancer surgery services

Training opportunities

Techniques/ methods that the student will have access to as part of the project:

- sample handling and preparation;

- atmospheric solids analysis probe mass spectrometry (ASAP-MS);

- (possibly, later in the project) desorption electrospray mass spectrometry (DESI-MS);

- machine learning and multivariate statistics methods applied to mass spectrometric data;

- coding in Python or Matlab;

- scientific writing and presentation skills

Ideal student background: The project is suitable for students from a wide range of backgrounds, particularly those from a chemistry or clinical background or with an interest in applying machine learning approaches to clinical medicine. The project involves mass spectrometric measurements on clinical biopsy samples and significant amounts of data analysis, employing both statistical methods and machine learning. An interest in

instrumentation is essential, as some technique development and optimisation will be required. Previous coding experience is not essential – most students who join our group learn this as they go along - but would be helpful.

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42. Targeting Nucleolin for Cancer Imaging and Therapy – Katherine Vallis

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

Abstract of the project

The nucleolus, a sub-nuclear structure, is the site of RNA biogenesis and is also involved in DNA damage repair, cell cycle control and stress responses. Nucleolin (NCL), a highly conserved phosphoprotein, is a major constituent of the nucleolus and has been shown to contribute to tumour growth, metastasis and drug resistance. NCL is found not only in the nucleolus but also in the nucleoplasm, cytoplasm and, sometimes, at the cell surface. NCL undergoes post-translational modifications that determine its localisation and function.

It has been reported that cell surface expression of NCL is especially prominent in cancer and in tumour-associated endothelial cells. We have recently confirmed that NCL is overexpressed at the plasma membrane in a large panel of cancer cell lines (including pancreatic, breast, prostate and oesophageal cancers) in comparison to relevant non-malignant cells. Interestingly we have also shown that nucleolin translocates to the nucleoplasm in hypoxia[1] and that cell surface expression is induced in hypoxic conditions (unpublished). The role of NCL at the cell surface is ill understood but it is thought to be involved in cell adhesion and extracellular signalling. What is known is that on binding a range of different ligands the NCL-ligand complex internalises into the cytoplasm. It is this feature that we plan to exploit in the current project, the central aim of which is to develop NCL-specific theranostic agents for the imaging and treatment of cancer.

Research objectives and proposed outcomes

In preliminary work our collaborators at the RFI Nanobodies Discovery Platform have generated a nanobody (single chain camelid antibody) against human NCL which will act as our prototypic carrier molecule[2]. Other antibody formats and peptide binders of NCL will be developed and evaluated. (**1**) The first objective will be to investigate the specificity and binding characteristics of an NCL-targeting nanobody in cells that express NCL at the cell surface versus those that do not or in which NCL has been knocked down, using a range of approaches including confocal fluorescence microscopy, live cell imaging, flow cytometry and saturation and competitive binding approaches. The initial focus will be on testing in breast cancer models, although other cancer types may be included as the project progresses. (**2**) In addition to antibody-based vehicles for targeting NCL, the project will include a peptide screen using a phage display approach to identify peptide binders to cell surface displayed NCL. (**3**) Protocols will be developed for radiolabelling of candidate carrier molecules with imaging and therapeutic radionuclides, and *in vitro* experiments performed to confirm retained binding properties. (**4**) The project will involve *in vivo* investigations to test the biodistribution and pharmacokinetics of candidate radiolabelled NCL-binders in molecular imaging (PET- or SPECT-CT) and tumour growth inhibition studies in appropriate xenograft, syngeneic and orthotopic models.

Academic value of the research project

This project will generate new knowledge about the extent and ubiquity of cell surface expression of NCL in cancer, lead to the discovery of high affinity NCL-binding molecules and will provide preclinical evidence regarding the suitability of NCL as a target for radiotheranostics.

Protein Production Facility at the Rosalind Franklin Institute. Prof Owen's background includes experience in industry as well as academia and is a leading authority on protein and antibody engineering.

Translational potential of the project

The central goal of this project is to develop theranostic agents for the imaging and treatment of cancer. Molecular imaging plays an increasingly important role in cancer medicine: in the selection of patients for precision oncology treatments and in monitoring response to treatment. New radionuclide therapeutics have

had enormous impact on the treatment landscape for prostate cancer and neuroendocrine tumours in recent years and there is a strong academic and industry drive to develop new agents[3]. Therefore, this project is timely, with a clear sightline to eventual clinical translation.

Training opportunities

This is an interdisciplinary project that relies on a new collaboration between the Departments of Oncology, the Nuffield Department of Surgical Sciences, the Rosalind Franklin Institute and the Nuffield Department of Medicine. Successful candidates will benefit from training in cell culture, molecular biology, advanced microscopy, antibody and peptide selection and engineering, molecular imaging, radiopharmaceutical development and statistical analysis. They will join a multidisciplinary research team with a strong track record of collaborative translational research and state of the art laboratory facilities.

Ideal student background: Candidates should have some basic experience and understanding of cancer biology and of the use of biologics (antibody and peptide) in cancer research.

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43. Melanin – at the heart of energy sensing – Richard White

Primary Supervisor: Richard White
Additional Supervisors: Colin Goding
Eligibility: Track 2 applicants are eligible to apply for this project

Abstract of the project
Cells must sense not only their internal energy state, but also the energy in their external environment. In most organisms, sunlight represents the most ubiquitous form of external energy. How cells and organisms sense sunlight, and respond to it, remains poorly understood. One of the key sensors of sunlight is melanin, the pigment produced by melanocytes in skin. These are the cells that gives each of us our unique hair and skin color. Melanin is best known for absorbing and protecting us against the damaging effects of ultraviolet (UV) light. When this protection goes awry, melanocyte can transform into a cancer called melanoma. Despite this obvious role of melanin, however, melanin absorbs far outside of the UV spectrum. Instead, it absorbs sunlight across the entire electromagnetic spectrum, ranging from UV to visible to infrared. We understand very little of the physiological reasons for this broad absorption. Therefore, the goal of this D.Phil project is to dissect the consequences of melanin's absorption of the entirety of sunlight, and ask what potential role it plays in homeostasis vs. cancer. We will use a combination of in vitro (human iPS cells) and in vivo (zebrafish) to study this problem.

Research objectives and proposed outcome
With reference to (i) the academic value of the research (ii) the collaborations involved and how these will be facilitated by the award. The main goal of this project is to understand how and why melanin absorbs the entirety of the electromagnetic spectrum of sunlight. Our hypothesis is that melanin acts as a fundamental energy sensor, transmitting information about the external energy environment to the interior of the cell. To test this idea, we will use genetic and biochemical techniques. We and others have identified many genes putatively involved in melanin synthesis. These genes will be systematically examined for their effects on melanin synthesis. These studies will be coupled with RNA-seq, metabolomics and biochemical assays to understand how the cell responds to sunlight in the presence or absence of melanin. By studying this in isolated melanocytes as well as within the context of the whole animal, we can discern what is a cell-intrinsic effect versus a cell-extrinsic effect. Once we understand the core groups of genes involved, we will then ask whether these genes also play a role in melanoma, an aggressive skin cancer derived from the melanocyte. This work will be enabled by our prior development of numerous forms of melanoma in the zebrafish. Collectively, this project aims to connect a biophysical property of melanin (energy sensing) to its broader physiologic role in health and disease.

Translational potential of the project
This project has immediate translational implications. We will identify genes involved in melanin synthesis, and how they relate to organismal homeostasis vs. cancer. Many of these genes are known to harbor single nucleotide polymorphisms (SNPs) across humans of varying skin tones, with concomitant varying risks of melanoma. Thus, our studies have particular relevance for cancer prevention.

Training opportunities
The student will receive rigorous training in genetic methods (CRISPR, genome editing, genetic screens), phenotypic readouts (animal and cell pigmentation), transcriptomics (single-cell and bulk RNA-seq), cell culture, and zebrafish husbandry. They will become expert in melanocyte, melanin and melanoma biology using both human iPS cells as well as zebrafish.

Ideal student background: Genetics, biochemistry, material science, engineering, physics

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44. Targeting the local and systemic mechanisms of immune suppression driven by liver metastases – David Withers

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

Abstract of the project
Despite the improvement in cancer treatment achieved through immune checkpoint blockade (ICB), durable curative responses are realised in only a small minority of patients with certain cancers[1-3]. This is best evidenced in colorectal cancer (CRC), where only the small subset of patients with deficient mismatch repair disease receives real benefit from treatment with ICB, particularly if given prior to surgery[4]. CRC is the 3nd most common cancer worldwide and frequently caught late. For late-stage disease, five-year survival figures remain awful (~10%). A fundamental challenge in treating late-stage CRC is the high prevalence of liver metastases, which strongly associate with poor response to treatment and survival. Even in typically ICB-responsive cancers such as melanoma or non-small cell lung cancer, the outcomes for patients with liver metastases are significantly worse[5-7]. Thus, liver metastases present a key barrier to treating many cancer patients with ICB and the critical obstacle for treating late-stage CRC. Of note, treatment failure is not limited to progression of the liver disease, but systemic progression. Understanding why and how liver metastases impede both the local and systemic anti-tumour response is vital to support the design of better treatments combinations for CRC, but highly relevant for late-stage disease in other cancer types.

Dissecting the mechanisms underlying the suppressive effect of liver metastases on systemic anti-tumour immunity. requires complex *in vivo* models. Utilising dual implantation of tumour cells intrahepatically and intradermally, we and others[8,9] have observed that tumour growth within the liver impairs tumour control at the intradermal site **(Fig.1).** Impaired systemic anti-tumour immunity has been attributed to the loss of antigen-specific CD8 T cells, due to tumour-associated macrophage populations within the liver[8]. However, this observation was not replicated in other studies[9]. Rather, suppression was linked to regulatory CD4 T cells (Tregs). Building on the pioneering photo-labelling approaches developed to assess immune dynamics in tumours[10-12], we have observed the traffic of Tregs from liver tumours to intradermal tumours and their draining lymph nodes **(Fig. 1)**, suggestive of a contribution of Tregs to the systemic immune suppression.

Here, we hypothesise that liver metastases: a) *mediate systemic immune suppression resulting in inhibiton of the anti-tumour response within an anatomically distant tumour* and b) *this suppression is mediated by the direct cellular traffic of regulatory cells from the liver to other tumour sites and their draining lymphoid tissue.*

Deciphering how and why liver metastases orchestrate systemic immune suppression has the potential to support the design of immunotherapy combinations tailored to ensure robust and enduring T cell responses. The hepatic intratumoural Treg compartment may be a promising target to disrupt, providing a potential route to tailored immunotherapy combinations appropriate for CRC patients with metastatic liver disease.

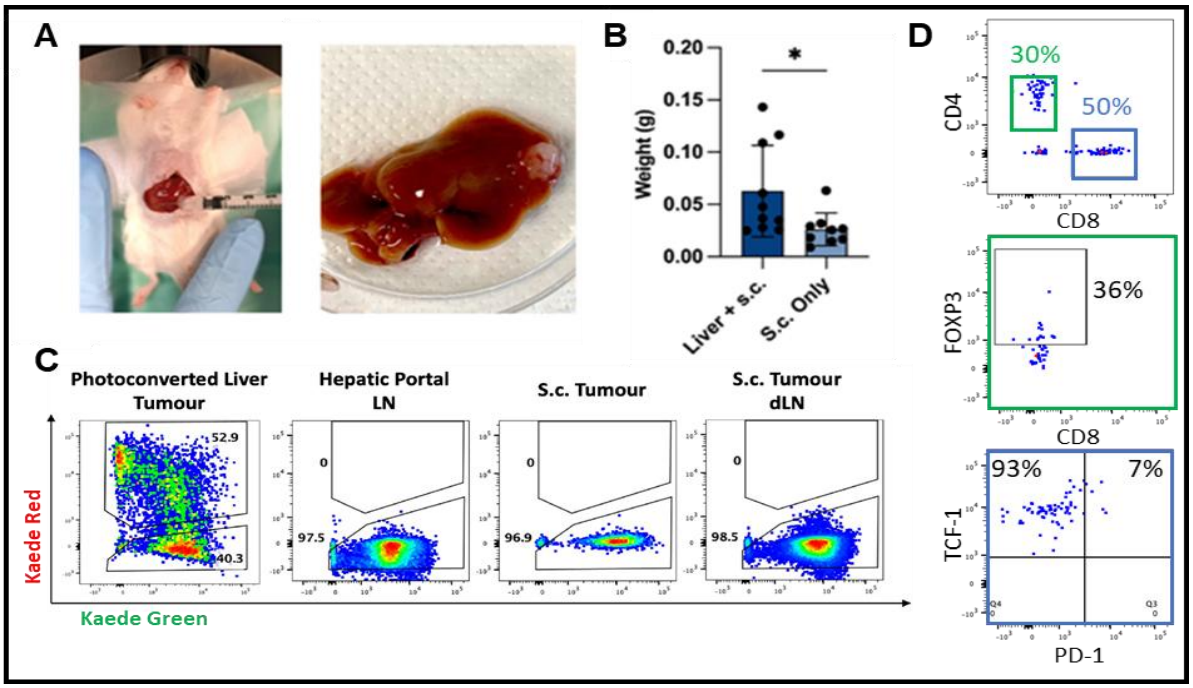

Figure 1. In vivo models to assess immune cell traffic from liver tumours. *(A) Image of intrahepatic injection of tumour cells and subsequent growth of single liver tumour. (B) Weights of s.c. tumours when liver tumours additionally present (liver + s.c.) or not (s.c. only). (C) Flow cytometry plots showing successful targeted photolabeling of liver tumours in Kaede mice. (D) Flow cytometry plots showing Kaede red+ T cells in peripheral lymphoid tissue after photolabeling of liver tumours. Data indicate that Tregs migrate to the lymph node draining subcutaneous tumours.*

To investigate the mechanisms driving immune suppression, we will firstly interrogate differences in the immune compartment of peripheral (intradermal) tumours in the presence and absence of intrahepatic tumours, using multiple approaches including sequencing (bulk-seq, scRNA-seq), spectral flow cytometry and immunofluorescence. Direct cellular traffic between tumours and draining lymphoid tissue will be interrogated *in vivo* using established photo-labelling approaches. Functional tests of trafficking populations will be performed alongside analysis of mechanisms mediating dissemination. More complex *in vivo* CRC models that include natural metastasis to different sites (liver, lung) will be utilised as the project develops to ensure translational relevance. Building on these data immunotherapeutic combinations to target these immunosuppressive mechanisms will be tested *in vivo* and their ability to improve tumour control in the context of liver metastases determined.

Collectively these experiments will provide an exciting and challenging DPhil project that sits at the cutting-edge of investigating the key mechanisms to target to refine cancer treatments and improve patient outcomes.

Research objectives and proposed outcomes

The specific research objectives for this project are as follows:

1. Characterise and compare differences in the immune compartment of different mouse models of primary and metastatic CRC.
2. Directly assess the immune traffic between different tumours to better understand immune cell dissemination and potential mechanisms underpinning immune suppression.
3. Test the functional role of immune cells trafficking to peripheral tissues as well as local populations in the liver in suppressing anti-tumour immunity.

4. Develop approaches to target the identified immune suppressive mechanisms within models of hepatic metastasis and enhance the anti-tumour immune response.

Translational potential of the project
Liver metastases represent one of the greatest barriers to the successful treatment of late-stage CRC. Understanding of the mechanisms operating in liver metastases are of further relevance to treatment failure across multiple other cancer types. Insight into how systemic immune suppression is orchestrated can support more targeted immunotherapy combinations to be developed. While this is a fundamental discovery research project, it aims to help address a critical knowledge gap and aid the development of better therapies for the many CRC patients that currently lack any viable options.

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. Systemic immune responses, capturing interactions within and trafficking across multiple tissues, can only be fully interrogated *in vivo*. Extensive training in an array of cutting-edge experimental approaches (e.g. photo-labelling, conditional targeting of molecular interactions and fate-mapping) across multiple cancer models (intradermal, orthotopic and mouse-tumour organoid) will be provided. Core lab skills of cell culture, transcriptomic analyses (e.g. scRNA-seq), flow cytometry (including spectral flow) and immunofluorescence imaging of tissue will form the backbone of the project, further complimented by *ex vivo* functional experiments and bioinformatics analyses. Collectively this will ensure comprehensive training in the core approaches required to interrogate anti-tumour responses, with appropriate appreciation of immune cell phenotype, spatial distribution, cellular interactions and functional relevance.

This research project benefits hugely from the combined expertise of the Withers and Barnes Labs as well as close interactions with multiple other groups: Dr Alex Gordon-Weeks (Oncology), Dr Felipe Galvez-Cancino (NDM-CIO) and Professor Simon Leedham (Oncology). This project complements the research focus of a CRUK Programme Award (led by DRW) and the student will be fully integrated into the regular meetings underpinning this research programme. The Withers Lab is highly collaborative and adopts a team science approach. *In vivo* research in the Withers Lab benefits from a dedicated and highly experienced technician in the lab. The lab has a long track record in providing a supportive environment in which post-graduate students flourish.

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

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45. Aspirin enhances cancer immunotherapy by releasing T cells from suppression by thromboxane – Jie Yang

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

Abstract of the project
Releasing T cells from immune suppression by targeting immune checkpoints such as CTLA-4 and PD-L1 results in effective clinical responses in some patients with cancer. However, only a minority of patients with a subset of cancers durably respond to existing immunotherapies. Failure to durably respond is referred to as immunotherapy resistance. While immunotherapy resistance is associated with low levels of lymphocytic infiltration and poor neoantigen load, large residual variability in clinical responses points to additional suppressive mechanisms limiting immunotherapy responses[1,2]. There is a need to identify and therapeutically target distinct mechanisms of immunosuppression if we are to build upon early successes in the field of cancer immunotherapy for the benefit of the majority of patients who presently do not respond. This proposed research has the potential to significantly improve patient outcomes by enhancing the efficacy of existing immunotherapies and developing new targeted approaches.

G protein-coupled receptors (GPCRs) play critical roles in cellular responses to extracellular signals. GPCRs are important drug targets - approximately 34% of all FDA-approved drugs target GPCRs. Our recent research has discovered a novel inhibitory pathway involving thromboxane A2 (TXA_2) and its receptor – a Gα12/13-coupled GPCR that limits T cell effector functions and anti-metastatic immunity[3,4]. We have found that the arachidonic acid metabolite TXA_2 acts via ARHGEF1, a guanine nucleotide exchange factor to limit kinase signalling and T cell effector functions[3,4]. Importantly, we have demonstrated that limiting TXA_2 availability using aspirin and other cyclooxygenase (COX)-1 inhibitors augments anti-metastatic immunity by limiting activation of the ARHGEF1 pathway in T cells, opening new avenues for therapeutic interventions[3,4].

Research objectives and proposed outcomes
Aim 1. Can we target the TXA_2 pathway to enhance cancer Immunotherapy?

Rationale: We found that TXA_2 signalling functions within T cells to suppress anti-metastatic immunity. We will determine how this pathway suppresses antigen-specific T cell responses to cancer and examine the therapeutic potential of targeting the pathway in combination with existing immunotherapies.

Strategy
The student will combine cutting-edge mouse cancer models, including orthotopically-implanted GEMM-derived organoid models, with newly-developed conditional mouse genetics tools to investigate the earliest adaptive immune responses during cancer metastasis, when metastasising cells, deprived of their immunosuppressive microenvironment, are most vulnerable to immune attack. The student will measure the impact of COX-1 inhibitors including **aspirin alone or in combination with existing immunotherapies** on various preclinical mouse cancer models, including colorectal and melanoma cancers, and analyse both the magnitude and phenotype of antigen-specific T cell responses within the tumour microenvironment. In addition, the student will explore whether the genetic ablation or pharmacological inhibitors targeting the TXA_2 pathway can augment adoptive T cell immunotherapy and CAR-T cell treatments for both metastasis and solid tumours. **High-dimensional flow cytometry** using marker panels established within the laboratory will be used to examine the differentiation state of bulk and antigen-specific $CD8^+$ T cell responses at high resolution using Cytek Aurora spectral analyser. This research will be employing **single-cell RNA sequencing** (scRNA-Seq) coupled with **TCR-sequencing** to dissect polyclonal T cell responses, focusing on the transcriptional profiles, clonal diversity, and functional states of T cells in response to tumour antigens.

Aim 2. What are the molecular mechanisms by which TXA_2 suppresses T cell signalling and function?

Rationale: our recent work has revealed a complex signalling network through which TXA_2 suppresses T cell activation and effector function, highlighting the pleiotropic effects of TXA_2 on kinase pathway activation downstream of TCR signalling in T cells[3]. However, the full extent of this signalling network and its implications for T cell function in various disease contexts remain to be elucidated.

Strategy
The student will use cutting-edge **phosphoproteomic** and **transcriptomic** techniques and **CRISPR-based mutagenesis** to comprehensively map how TXA_2 controls T cell signalling and effector function in both mice and humans. These analyses will reveal novel insights into pathway regulation and identify potential therapeutic targets. The student will compare the pathway's operation in mouse and human T cells, identifying conserved and divergent mechanisms. Together, the student will use bioinformatics approaches to integrate the phosphoproteomic, transcriptomic, and functional data, creating a comprehensive model of TXA_2 signalling in T cells. This computational analysis could reveal novel insights into the pathway's regulation and function, potentially identifying new therapeutic targets.

Translational potential of the project
This research has promising translational potential with strong relevance to cancer patient care. Targeting the TXA_2 pathway using readily available COX-1 inhibitors, including aspirin as adjuvant immunotherapy, offers clear potential for enhancing immune checkpoint blockade efficacy. The availability of these widely-used, low-cost and accessible drugs enables rapid clinical translation, potentially transforming outcomes for the majority of advanced cancer patients who currently do not respond to immunotherapy. This fundamental research addresses a critical knowledge gap by identifying how TXA_2 suppresses T cell function in the tumour microenvironment. The pathway's involvement across multiple cancer types, combined with the wide availability of aspirin, positions this work to have immediate and widespread clinical impact, particularly valuable in resource-limited settings where expensive targeted therapies are not accessible.

This research is particularly timely and relevant to the aspirin and cancer field, as there are ongoing attempts to bring about changes to medical practice with new randomised controlled trials showing efficacy of low-dose aspirin in primary Lynch syndrome colorectal cancer prevention (**CaPP3**) and recurrence (**ALASSCA**)[5], alongside the ongoing **Add-Aspirin** trial (https://www.addaspirintrial.org/). In addition, this research aligns closely with current Oxford cancer prevention efforts such as the **LynchVax** programme. Our work on TXA_2-mediated T cell suppression could significantly enhance vaccine efficacy by removing immunosuppressive brakes on T cell responses. By combining aspirin treatment with cancer vaccines like LynchVax, we could potentially create synergistic prevention strategies where aspirin enhances T cell function while vaccines provide targeted antigen recognition.

Training opportunities
This project will provide excellent training opportunities for students who are passionate about cancer immunotherapy research. Students will gain expertise in mouse primary and metastatic tumour models, and advanced cellular and molecular immunology techniques including single-cell RNA-seq, spectral flow cytometry, CRISPR-based mutagenesis and mass spectrometry. Computational biology training will enable integration of phosphoproteomic, transcriptomic, and functional datasets using advanced bioinformatics approaches. Students will benefit from Oxford's highly collaborative environment with access to both the Department of Oncology and the Center for Immuno-Oncology (CIO). This unique opportunity provides exceptional exposure to cutting-edge immuno-oncology research spanning fundamental immunology to clinical translation with opportunities for professional development through conference presentations and high-impact publications, preparing them for leadership positions in academic research.

Ideal student background: The student requires basic knowledge in immunology. An enthusiasm and interest in cancer immunology is essential. Knowledge and experience in tumour immunology is obviously advantageous.

Experience in the use of immune assays, alongside in vivo or ex vivo experiments is desirable. The student should be willing to work with mouse models of cancer. Some prior bioinformatics experience or willingness to learn bioinformatics is important.

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