DPhil in Cancer Science – 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. We are currently open to applications on Track 2 for medical undergraduates who will **undertake a 3-year research project selected from this advertised 'DPhil in cancer science – Medical Undergraduate booklet'.**

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 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 stipend, University/College fees, and a research consumables budget.

Stipend provisions are summarised below:

- **Application Track 2:** 3 years of stipend at £22,123, October 2026.

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.

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 Friday 1st May.** It is strongly suggested that students contact supervisors of projects they are interested in applying for prior to application.

Projects

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

Contents

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

Primary Supervisor: Ivan Ahel
Additional Supervisors: Dragana Ahel
Eligibility: Track 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.

References

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2. 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 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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Phenotypic screen of sixty-eight colorectal cancer cell lines identifies CEACAM6 and CEACAM5 as markers of acid resistance. Michl J, White B*, Monterisi S, Bodmer WF, Swietach P. Proc Natl Acad Sci U S A. 2024 Mar 26;121(13):e2319055121. doi: 10.1073/pnas.2319055121. Epub 2024
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3. 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 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.

References

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

Primary Supervisor: Jason Davis
Additional Supervisors: Christiana Kartsonaki
Eligibility: 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.

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

Primary Supervisor: Fumiko Esashi
Additional Supervisors: Bass Hassan
Eligibility: 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.

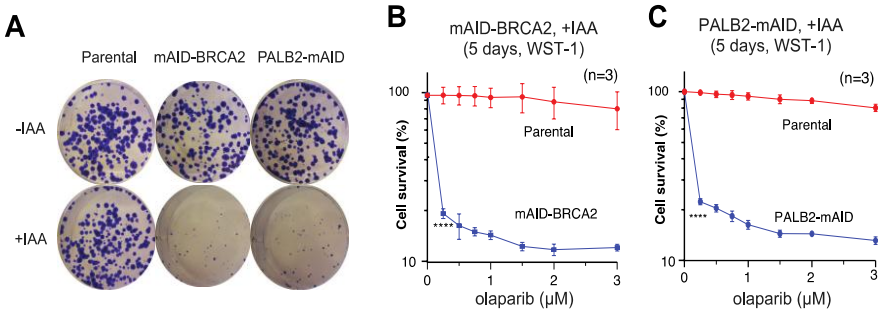

Figure 1. A. HCT116 mAID-BRCAs/PALB2-mAID cells were seeded in 6-well plates, and grown for 10 days with and without auxin (IAA). Colonies were then fixed and stained with crystal violet. **B-C:** HCT116 mAID-BRCA2/PALB2-mAID and parental HCT116 cells were first treated with IAA for 2 hours, and subsequently 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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6. 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 enthusiastic, reflective, highly-organised, tenacious and patient. The group-leader is happy to discuss the project informally with any potential applicants.

References

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Return to Projects list

7. 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 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.

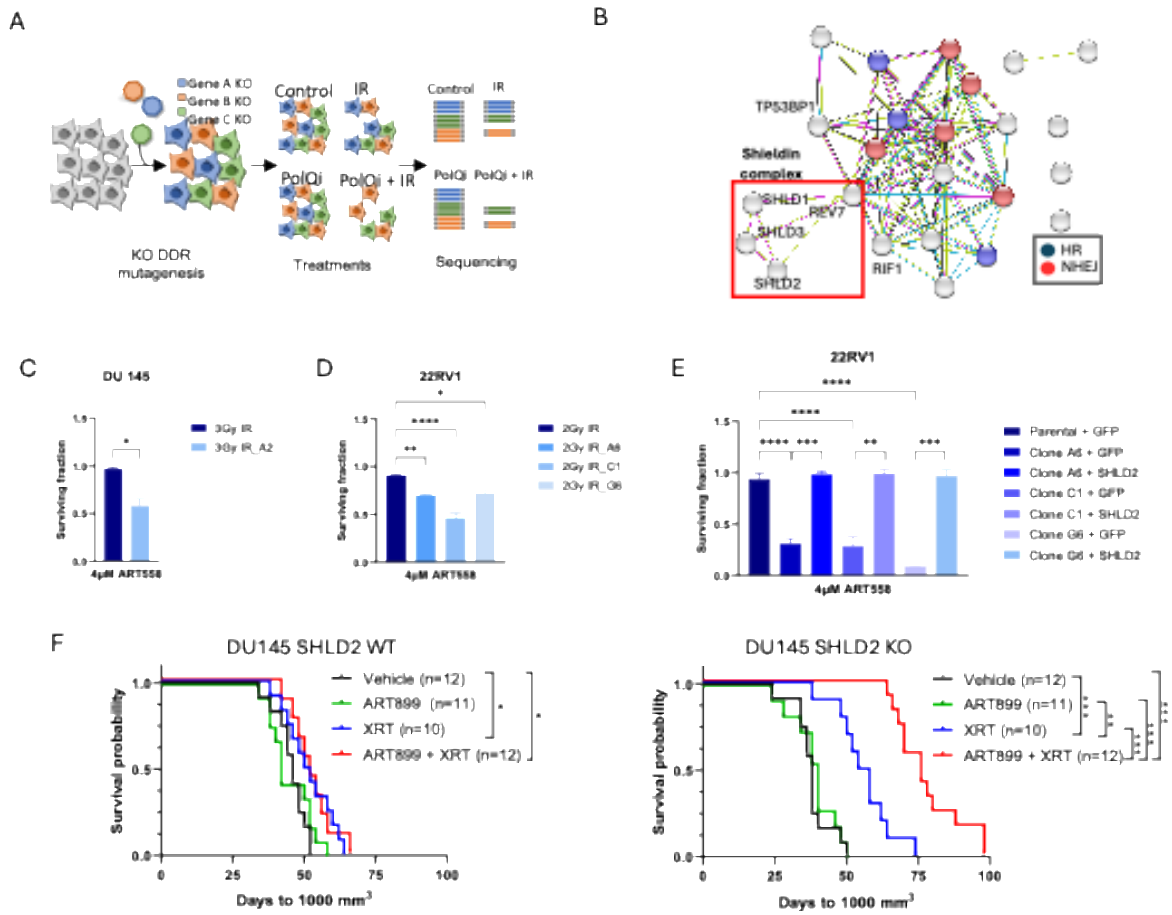

Figure 1. **(A)** Schematic of the DDR CRISPR screen in DLD1 cells. **(D)** Functional association networks of genes whose knockout caused significant radiopotentiation by Polθi in the DDR CRISPR screen (BioGrid) **(C)** Surviving fractions from colony formation assays of DU145 SHLD2 WT (parental) *vs* 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.

References

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8. 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 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.

References

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

Primary Supervisor: Peter J. McHugh
Additional Supervisors: Christopher J. Schofield
Eligibility: 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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10.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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11. Linear accelerator for Mega Voltage Photon FLASH radiotherapy – Krisoffer Petersson

Primary Supervisor: Kristoffer Petersson
Additional Supervisors: Geoff Higgins
Eligibility: 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.

12. 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 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.

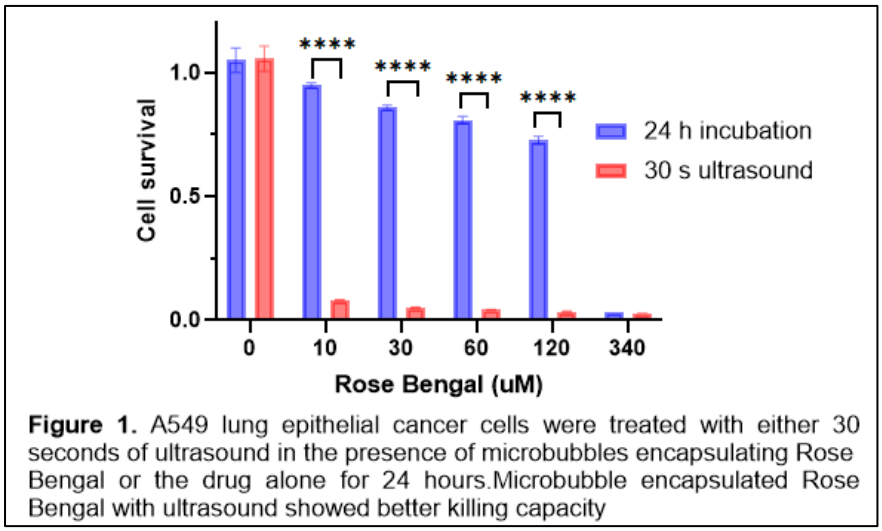

Figure 1. A549 lung epithelial cancer cells were treated with either 30 seconds of ultrasound in the presence of microbubbles encapsulating Rose Bengal or the drug alone for 24 hours.Microbubble encapsulated Rose Bengal with ultrasound showed better killing capacity

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.

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13.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 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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14. 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 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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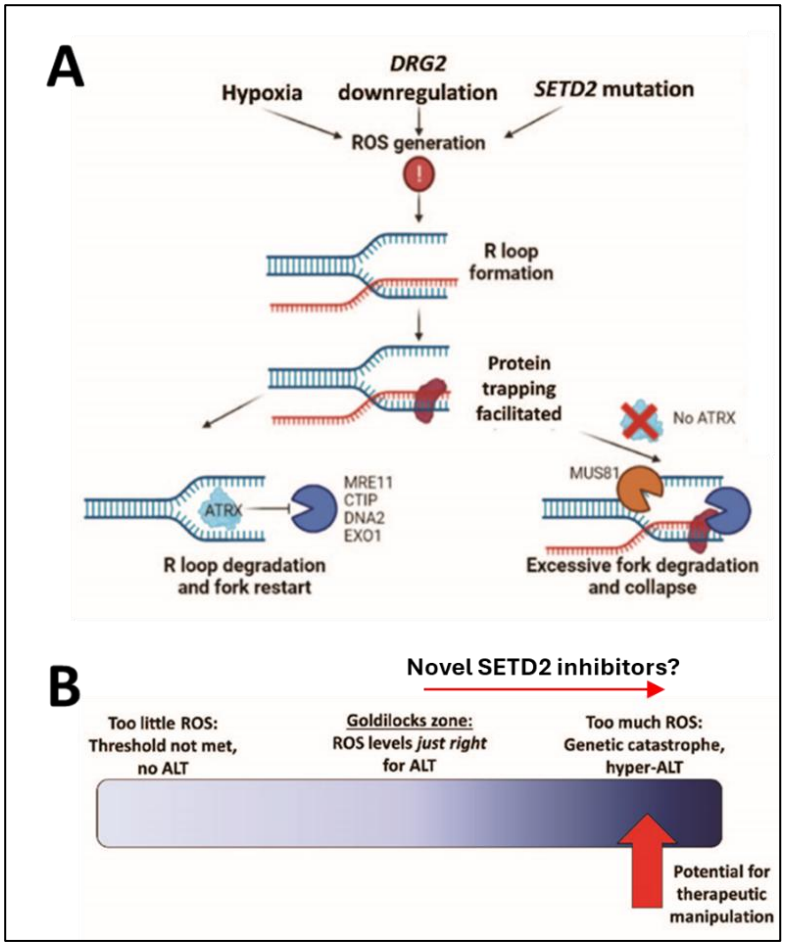
15. Exploring epigenetic changes in paediatric high-grade gliomas with SETD2/H3F3A mutations – Anna Rose

Primary Supervisor: Anna Rose
Additional Supervisors: Ester Hammond / Marketa Tomkova
Eligibility: Track 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.

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16. 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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17. 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 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.

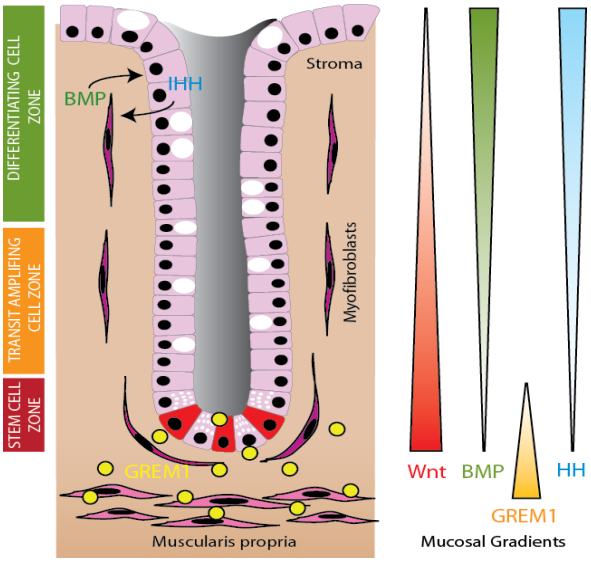

Fig. 1. Signalling regulation of intestinal homeostasis

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.

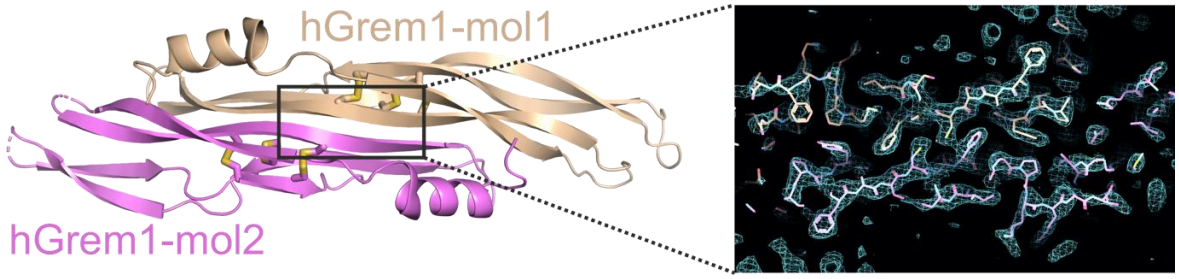

Fig.2: Crystal structure of our GREM1 dimer with experimental map shown in inset (unpublished). The purified protein sample used for crystallisation is able to stimulate organoid growth and efficiently inhibits BMP signalling in cells. This crystallization condition will form the basis of our planned 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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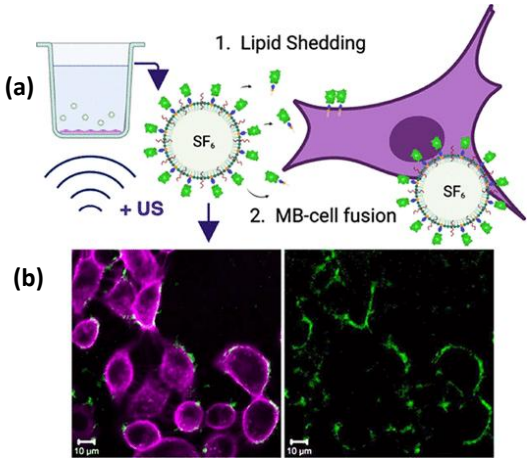
18. Ultrasound triggered cancer cell-tagging for radioimmunotherapy in glioblastoma multiforme – Eleanor Stride

Primary Supervisor: Eleanor Stride
Additional Supervisors: Edward O'Neill
Eligibility: 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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19. Beyond tumour control: Investigating brain aging after checkpoint immunotherapy for melanoma – Anya Topiwala

Primary Supervisor: Anya Topiwala
Additional Supervisors: Benjamin Fairfax
Eligibility: 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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20. 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 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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21. Aspirin enhances cancer immunotherapy by releasing T cells from suppression by thromboxane – Jie Yang

Primary Supervisor: Jie Yang
Additional Supervisors: David Withers
Eligibility: 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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