DPhil in Cancer Science

University of Oxford

GSK Studentships

2025 Intake Project Booklet

OXFORD CANCER

UNIVERSITY OF OXFORD

NHS
Oxford University Hospitals
NHS Foundation Trust

GO-PRECISE Studentship Project Booklet

Introduction

This handbook provides an overview for prospective students looking to apply for a GO-PRECISE DPhil as part of the GSK funded studentships.

Global biopharma company GSK is investing up to £50 million in a collaboration with Oxford to advance the understanding of how cancer develops, which could inform future development of vaccines to prevent cancer. The agreement establishes the GSK-Oxford Cancer Immuno-Prevention Programme, aimed at exploring the potential of cancer prevention through vaccination.

The programme leverages the complementary expertise of GSK and Oxford in the science of the immune system, vaccine development and cancer biology. It is hoped that the insights generated through the programme into how cancer develops could inform new approaches to vaccination for cancer prevention, offering fresh hope in the fight against the disease.

As part of the programme, there are three studentships available across seven projects for applicants to apply to.

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

Successful applicants will enrol on a four-year programme with the first year consisting of two six-month rotations within the selected project labs. This provides students with a broad base of experience and the opportunity to explore different aspects of research. All students are admitted directly to work under the supervision of a Principle Investigator (PI).

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

All applicants will be judged on the following:

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

Funding

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

- Four years of stipend at the flat rate of £21,000 per annum.

How to Apply

A detailed summary on how to apply can be found here. In brief, prospective students apply with a **prioritised list of three projects selected from this booklet by Midday on Friday 25th April.**

Shortlisted students will be invited to interview in May. If successful, students will then begin their first year with two lab rotations. It is strongly suggested that students contact supervisors of projects they are interested in applying for prior to application.

Projects

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

Contents

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

Primary Supervisor: Sarah Blagden
Additional Supervisors: Roman Fischer

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

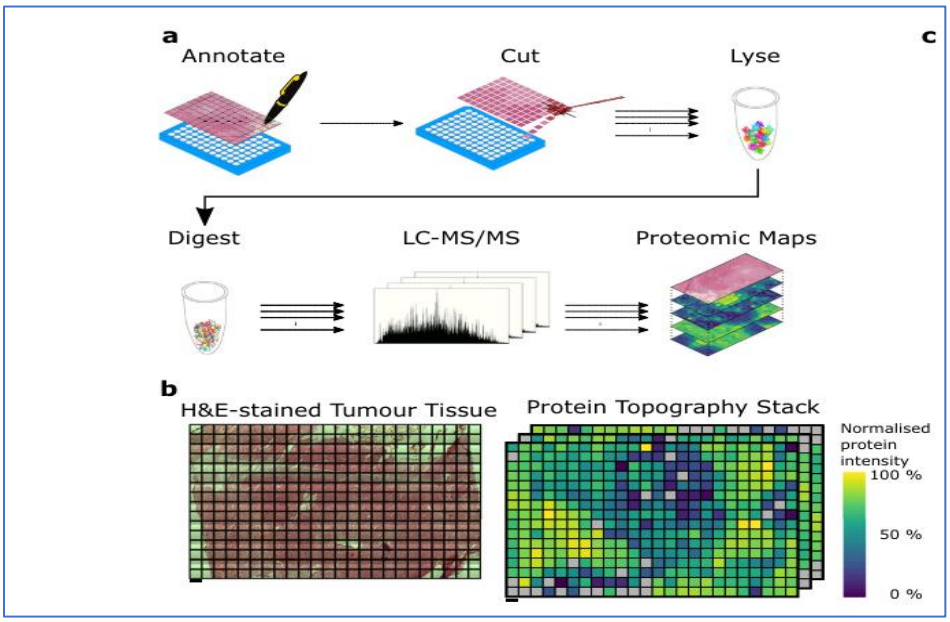
Prof Roman Fischer is the UK's foremost expert in a new technique called LCM-LCMS that combines tumour laser capture microdissection (LCM) with topographic proteomics (LC-MS), enabling detailed characterisation of over 5,000 proteins within individual cells or microscopic regions of tissue. He has optimised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

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

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

Research objectives and proposed outcomes
The project will comprise of four **work packages (WPs).**

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



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

WP2: Validate candidates by IHC using existing tissue microarrays (developed by the Translational Histopathology Lab at ORCRB) containing samples of normal, STIC lesions and HGSOC tissue. Explore the impact of CRISPR-cas9 target depletion in ovarian cancer cell lines and in ovarian organoid models.

WP3: Assay circulating plasma from normal, cancer and precancer patients for levels of identified markers using immune-precipitation/sandwich ELISA. Identify shortlist of candidates to develop PRM mass spec method for detecting constituent peptides in plasma.

WP4: Verify biomarkers in wider set of plasma samples collected longitudinally prior to ovarian cancer diagnosis.

Translational potential of the project

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

Training opportunities

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

References

Labidi-Galy SI, Papp E, Hallberg D et al. High grade serous ovarian carcinomas originate in the fallopian tube. Nat Commun. 2017 Oct 23;8(1):1093. doi: 10.1038/s41467-017-00962-1.

Menon U, Gentry-Maharaj A, Burnell M, et al. Ovarian cancer population screening and mortality after long-term follow-up in the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS): a randomised controlled trial. Lancet. 2021 Jun 5;397(10290):2182-2193. doi: 10.1016/S0140-6736(21)00731-5

Davis S, Scott C, Oetjen J, Charles PD, Kessler BM, Ansorge O, Fischer R. Deep topographic proteomics of a human brain tumour. Nat Commun. 2023 Nov 24;14(1):7710. doi: 10.1038/s41467-023-43520-8.

2. Discovering and targeting 'dark antigens' in cancers and precancers – Sarah Blagden

Primary Supervisor: Sarah Blagden
Additional Supervisors: Tim Elliott

Abstract of the project
Approximately 1:400 women carry BRCA1 and BRCA2 mutations and have a 20-50% and 40-85% lifetime risk of ovarian and breast cancer respectively and other cancers less commonly associated include pancreatic and prostate cancer. The current standard-of-care cancer preventive treatment for BRCA carriers is risk-reducing removal of breasts (mastectomy) at 30 years, and of ovaries and fallopian tubes (salpingo-oophorectomy) at 40 years of age. Surgery, particularly the removal of ovaries, can lead to significant physical and psychological morbidity and, hence, many BRCA carriers decline it. We now know that breast and ovarian cancer do not develop quickly but arise over 4-6 years as cells transition from normal to invasive cancer. This latent stage is called "precancer". In BRCA carriers, ovarian precancers are called "serous tubal intraepithelial carcinomas" (STICs) and arise as microscopic collections of abnormal cells in the fallopian tubes that spread to the ovaries once they have transitioned to invasive cancer. Similarly, breast cancer starts as a precancer called ductal carcinoma in situ (DCIS).

It is likely that the vast majority of precancers are recognised by the body's immune cells, particularly CD8+ T cells which identify abnormal peptides (neoantigens) displayed on their cell surface by MHC class I molecules whereupon they engulf and destroy them. However, precancers that escape immune recognition can transition to invasive cancer, a process termed 'immune escape'. We hypothesise that a vaccine that specifically activates T cells to recognise the neoantigens presented by precancers could prevent them from escaping surveillance6,7. In Oxford, we are using immunopeptidomics to sequence MHC-presented neoantigens correlated with RNA sequencing to identify the transcripts coding for these peptides, and then applying AI algorithms to predict their expression by different HLA alleles.

While this technique works for some precancers, our preliminary findings indicate that BRCA-associated cancers present "non-canonical" or dark antigens (DAs), proteins encoded by normally silenced or lowly expressed regions of the genome, like transposable elements, endogenous retroviruses, or other non-coding regions. This is likely because BRCA1 and BRCA2, which are crucial for homologous recombination repair (HRR) of DNA double-strand breaks, when mutated cause genomic instability contributing to the reactivation of normally silenced genomic elements. In this case, RNA seq alone is inadequate for DA detection and long-read sequencing may be preferable. The aims of this project are three-fold.

The overarching purpose of this proposal is exploring DAs in BRCA-associated breast, ovarian, pancreatic and prostate cancers using immunopeptidomics, RNA seq and long-read sequencing (Oxford Nanonpore) to identified shared and clonal dark (non-canonical) as well as canonical neoantigens. These findings could inform a neoepitope-based vaccine to be given to BRCA carriers as a non-surgical, non-invasive cancer preventive.

Research objectives and proposed outcomes
The project will comprise of four **work packages (WPs).**

WP1. Work Package 1: Transcriptome Characterisation

Objective: To characterise mBRCA cancer cell lines (breast, ovarian, prostate and pancreatic) and tumour tissues for subsequent analyses of dark antigens.

1. Perform initial genomic and transcriptomic characterisation of cell lines and fresh cancer tissues using:
   Long-read sequencing: To identify full-length transcripts, alternative splicing events, and fusion genes.
   RNA-seq: To analyse gene expression profiles and quantify known and novel transcripts.

Work Package 2: Immunopeptidomics Analysis

Objective: To identify and characterise the MHC-bound peptides presented on cancer cells and tissues, focusing on dark antigens.

1. **Immunopeptide Isolation:** Isolate MHC-bound peptides from cancer cell lines and tumour tissues using immunoprecipitation with specific MHC antibodies.
2. **Mass Spectrometry (MS) Analysis:** Perform high-resolution mass spectrometry to analyse the isolated peptides and identify their sequences. Use software tools (e.g., MaxQuant, PEAKS) to match peptide sequences to known databases, including potential dark antigens.
3. **Data Integration:** Integrate MS data with RNA-seq and long-read sequencing results to link peptide identification with their corresponding transcripts and genes. Identify novel or cryptic peptides that arise from unannotated or non-canonical transcripts.

Work Package 3: Functional Characterisation of Dark Antigens

Objective: To validate the expression and immunogenicity of identified dark antigens in cancer models.

1. **Validation of Dark Antigen Expression:** Use quantitative PCR and western blotting to confirm the expression of identified dark antigens at the RNA and protein levels in cell lines and tumour tissues. Utilise LRS to validate the presence of alternative transcripts contributing to dark antigen expression.
2. **Functional Assays:** Conduct T-cell activation assays using isolated T cells from healthy donors or patients to test the immunogenicity of the identified dark antigens. Assess whether dark antigens elicit a measurable immune response, including cytokine production and T-cell proliferation.
3. **Bioinformatics Analysis:** Analyse expression data to correlate dark antigen expression levels with patient clinical outcomes and treatment responses. Explore potential associations between specific dark antigens and immune evasion mechanisms.

Work Package 4: Biomarker Discovery and Therapeutic Implications

Objective: To identify potential biomarkers for cancer immunotherapy and explore therapeutic applications of dark antigens.

1. Biomarker Identification: Apply statistical and bioinformatics tools to assess the significance and clinical relevance of identified dark antigens. Investigate the potential of identified dark antigens as targets for cancer immunotherapy (e.g., vaccine development, T-cell therapy).

2. PreClinical Validation: Validate the relevance of identified biomarkers using established mouse cancer models.

Translational potential

The earlier detection and prevention of cancer are central objectives of the University of Oxford and CRUK. If successful, the student will generate a data-driven pipeline to identify tumour-specific, non-canonical antigens for immunotherapy and vaccine development.

Training opportunities

This is an interdisciplinary project between the labs of Prof Sarah Blagden (cancer biology and clinical trials), Prof Tim Elliott (immunopeptidomics, cancer immuno-oncology and HLA prediction modelling) and with bioinformatics support from Andy Blake (dataset integration). Alongside basic wet lab techniques, the student will learn bioinformatic methods, culminating in multi-omics integration to generate a comprehensive DA database.

Return to Projects list

3. Analysis of the effects of novel cancer vaccines on the phenotype and function of human dendritic cells in individuals at high risk of lung cancer and lung cancer patients – Tim Elliott

Primary Supervisor: Tim Elliott
Additional Supervisors: Maria Aggelakopoulou

Abstract of the project
Optimal T cell immune responses against cancer dependent on dendritic cells (DCs), that present tumour-derived antigens to T cells and provide functional cues, such as costimulatory ligands and cytokines (1). Studies by our team and others support the hypothesis that mRNA-LNPs are taken up by specific DC subsets which are directly activated to mediate chemokine and cytokine release and drive an adaptive immune response, particularly favoring T follicular helper cell and humoral responses (2). However, our understanding about DC functions underlying the ability of cancer mRNA-LNP vaccines to induce anti-tumour T cell responses is limited. This research proposal aims to shed light on the effects of novel mRNA-LNP vaccines on the phenotype and function of DCs in individuals at high risk of lung cancer (pre-cancer) and lung cancer resected patients. Using mass spectrometry profiling, we will analyse the repertoire of MHC Class I- and Class II-bound peptides by DCs before and after treatment with different mRNA-LNP formulations. Also, single cell RNA sequencing, spectral cytometry, analysis of synapse formation and functional DC- T cell co-culture assays with be deployed to assess changes on the phenotype and functional states of DCs and how these correlate with the priming and maintenance of effective CD8 and CD4 T cell responses. These studies will provide significant insights for the rational design of mRNA -LNP vaccines with enhanced efficacy.

References

1) Luri-Rey C, Teijeira Á, Wculek SK, de Andrea C, Herrero C, Lopez-Janeiro A, Rodríguez-Ruiz ME, Heras I, Aggelakopoulou M, Berraondo P, Sancho D, Melero I. Cross-priming in cancer immunology and immunotherapy. Nat Rev Cancer. 2025 Jan 29. doi: 10.1038/s41568-024-00785-5. Epub ahead of print. PMID: 39881005.

2) Verbeke R, Hogan MJ, Loré K, Pardi N. Innate immune mechanisms of mRNA vaccines. Immunity. 2022 Nov 8;55(11):1993-2005. doi: 10.1016/j.immuni.2022.10.014. PMID: 36351374; PMCID: PMC9641982.

4. Establishing of human lung cancer organoids to study T cell responses to cancer mRNA-LNP vaccines – Tim Elliott

Primary Supervisor: Tim Elliott
Additional Supervisors: Maria Aggelakopoulou

Abstract of the project
Highly effective cancer vaccines should elicit specific, robust and durable T cell responses. To advance informed design of cancer mRNA-LNP vaccines, it is critical that we understand the cellular dynamics underlying responses to multiple epitopes, mRNA modifications and LNP formulations. However, studying the human immune system in vivo is challenging and often not possible. This research project aims to understand how antigen-specific CD8 and CD4 T cells are activated and participated in adaptive immune responses within the tumour site. We will optimise and use a human lung cancer organoid- T cell co-culture model to track the differentiation and kinetics of the adaptive immune response to different mRNA- LNP formulations. We will track the magnitude, diversity, phenotype and effector function of elicited CD8 and CD4 T cell responses, using spectral/flow cytometry, TCR sequencing and scRNA sequencing. Leveraging patient derived tumour organoids holds promise for advancing the rational design of effective mRNA -LNP vaccines.

Return to Projects list

5. Clonal T cell competition following vaccination – Tim Elliott

Primary Supervisor: Tim Elliott
Additional Supervisors: Ellie Barnes, Felipe Galvez-Cancino

Abstract of the project
Previously we have shown that CD8+ T-cells compete for nonspecific stimuli early after activation via a (DNA) cancer vaccine and prior to cell division. When high-affinity competitive CD8+ T-cells were deleted by self-antigen expression, competition was abrogated suggesting that limiting clonal T cell competition following vaccination might lead to broader CD8+ T cell responses (1). We will focus on the dynamics of T cell responses to multiple epitopes utilizing a mouse model immunized with a multi-epitope mRNA vaccine containing both dominant and subdominant antigens (2, 3). We will track the expansion and contraction of T cell clones specific to each epitope using high-throughput TCR sequencing and flow cytometry over several months post-vaccination and following challenge with a transplantable syngeneic tumour. The project will elucidate how different T cell clones compete for resources, such as antigen presentation on dendritic cells, and how this competition affects the overall immune response. Additionally, the study will explore how factors like antigen dose, adjuvant type, and booster immunizations influence the competitive landscape among T cell clones.

Research objectives and proposed outcomes
CD8+ T-cell responses to multi-epitope vaccines are shaped by competition dynamics influenced by peptide–MHC class I (pMHC) stability, antigen accessibility on dendritic cells (DCs), and pre-existing immunity. Galea et al. (2012) demonstrated that CD8+ T cells recognizing high-stability pMHC complexes dominate early clonal expansion by outcompeting others for nonspecific stimuli, a process reversible upon deletion of high-affinity clones. Johnson et al. (2016) revealed that memory CD8+ T cells primed by prior vaccination suppress naïve T-cell responses to heterologous antigens via competition for shared DCs, compromising protective immunity. Garcia et al. (2007) showed that T-cell–DC interaction stability depends on antigen availability, with high T-cell precursor frequencies intensifying competition for limited pMHC complexes. Together, these studies highlight how clonal competition restricts response breadth and efficacy. This project aims to exploit these mechanisms to design vaccination strategies that mitigate competition and enhance polyclonal CD8+ T-cell responses against cancer.

Work Package 1: Epitope Hierarchy and TCR Clonal Diversity
Objective: Define how pMHC stability and TCR affinity dictate clonal dominance in multi-epitope mRNA vaccines.
Approach: Immunize mice with mRNA vaccines encoding dominant (high pMHC stability) and subdominant (low pMHC stability) epitopes. Track clonal expansion/contraction using TCRβ sequencing and flow cytometry over 6 months post-vaccination. Correlate epitope-specific TCR diversity with pMHC stability using tetramer decay assays. Challenge mice with syngeneic tumours expressing vaccine epitopes to assess protective immunity breadth.
Prior Work: Builds on Galea et al. (2012) pMHC stability hierarchy and Garcia et al. (2007) T-cell–DC competition dynamics.

Work Package 2: Antigen Accessibility and Dendritic Cell Interactions
Objective: Determine whether segregating epitopes across DC subsets reduces clonal competition.
Approach: Administer mRNA vaccines with epitopes targeted to distinct DC populations (e.g., CD8α+ vs. CD11b+ DCs)57. Quantify epitope-specific T-cell expansion using intracellular cytokine staining and TCR sequencing. There may be an opportunity to use two-photon imaging to visualize T-cell–DC interaction stability in lymph nodes. Compare responses to vaccines with epitopes co-delivered versus split across DCs.

Prior Work: Extends Johnson et al. (2016) findings on antigen competition and Garcia et al. (2007) DC interaction dynamics.

Work Package 3: Modulation of Vaccine Parameters
Objective: Optimize antigen dose, adjuvant type, and boosting to broaden clonal responses.
Approach: Test low vs. high antigen doses with TLR (e.g., Poly I:C) vs. STING adjuvants.
Evaluate prime-boost regimens with epitope permutations to disrupt pre-existing dominance. Measure transcriptomic profiles of dominant vs. subdominant clones (scRNA-seq). Validate strategies in tumour-challenge models and assess neoantigen response breadth.
Prior Work: Integrates Galea et al. (2012) on affinity-driven competition and Johnson et al. (2016) on heterologous suppression.

Training opportunities
This proposal leverages prior insights into T-cell competition mechanisms to develop vaccination strategies that enhance polyclonal responses. The project will provide training in in vitro and in vivo immunology and vaccine design, including techniques such as primary T cell culture, spectral flow cytometry, transcriptomics (including single-cell RNAseq) and advanced microscopy.

References
1. Galea, I., Stasakova, J., Dunscombe, M.S., Ottensmeier, C.H., Elliott, T. and Thirdborough, S.M. (2012), $CD8^+$ T-cell cross-competition is governed by peptide–MHC class I stability. Eur. J. Immunol., 42: 256-263. https://doi.org/10.1002/eji.201142010
2. Johnson LR, Weizman OE, Rapp M, Way SS, Sun JC. Epitope-Specific Vaccination Limits Clonal Expansion of Heterologous Naive T Cells during Viral Challenge. Cell Rep. 2016 Oct 11;17(3):636-644. doi: 10.1016/j.celrep.2016.09.019. PMID: 27732841; PMCID: PMC5503750
3. Z. Garcia,E. Pradelli,S. Celli,H. Beuneu,A. Simon,& P. Bousso Competition for antigen determines the stability of T cell–dendritic cell interactions during clonal expansion, *Proc. Natl. Acad. Sci. U.S.A.* 104 (11) 4553-4558, https://doi.org/10.1073/pnas.0610019104 (2007).

6. Impact of different LNP formulations affect T cell immunodominance – Tim Elliott

Primary Supervisor: Tim Elliott
Additional Supervisors: Ellie Barnes**,** Maria Aggelakopoulou

Abstract of the project
This research proposal aims to investigate the impact of mRNA and lipid nanoparticle (LNP) formulations on T cell immunodominance. Recent studies have shown that LNP-based mRNA vaccines can elicit potent T cell responses and enhance humoral immunity (1). However, the specific effects of different LNP compositions and mRNA modifications on T cell immunodominance patterns remain unclear. We hypothesize that altering LNP components, such as ionizable lipids and fusogenic helper lipids, as well as mRNA modifications, will influence antigen presentation and subsequent T cell activation, potentially shifting immunodominance hierarchies (2). By systematically evaluating various LNP-mRNA formulations in both *in vitro* and *in vivo* models, we aim to identify optimal combinations that can selectively enhance desired T cell responses against specific epitopes. This research will provide valuable insights for the rational design of more effective mRNA vaccines and could have significant implications for improving vaccine efficacy against infectious diseases and cancer.

Research objectives and proposed outcomes
Emerging evidence highlights lipid nanoparticles (LNPs) as critical platforms for mRNA vaccine delivery, with composition-dependent effects on immune activation. Recent studies demonstrate that LNP formulations containing ionizable lipids (e.g., C12-200, cKK-E12), β-sitosterol, and fusogenic helper lipids like DOPE enhance antigen presentation in dendritic cells (DCs) and drive robust T cell proliferation and cytokine production (IFN-γ, TNF-α, IL-2). Concurrently, mRNA modifications such as N4-acetylcytidine (ac4C) by NAT10 and m6A methylation have been shown to regulate translation efficiency and degradation kinetics, directly impacting T cell expansion and differentiation. Despite these advances, the interplay between LNP composition, mRNA modifications, and T cell immunodominance hierarchies—the preferential targeting of specific epitopes during immune responses—remains unexplored. This knowledge gap limits the rational design of vaccines capable of directing T cells against high-priority epitopes in infections or cancer.

Work Package 1: Systematic Screening of LNP Formulations on Antigen Presentation
Objective: Identify LNP components that optimize DC uptake, mRNA translation, and epitope presentation.
Methodology: Test 20+ LNP variants with variable ionizable lipids (C12-200, cKK-E12), helper lipids (DOPE vs. cholesterol/β-sitosterol ratios), and PEG content. Transfect bone marrow-derived DCs (BMDCs) with OVA-mRNA LNPs and quantify activation markers (CD40, CD86), IL-12 secretion, and MHC-I/II epitope presentation using mass spectrometry. Rank LNPs by their ability to induce cross-presentation of immunodominant vs. subdominant epitopes.

Work Package 2: mRNA Modification Engineering for Epitope-Specific Translation
Objective: Determine how chemical modifications (ac4C, m6A) alter mRNA stability and ribosomal engagement to skew immunodominance.
Methodology: Engineer OVA-mRNA with site-specific ac4C (via NAT10 co-delivery) or m6A modifications and encapsulate in top-performing LNPs from Work Package 125. Use ribosome profiling and RNA-seq in BMDCs to correlate modification patterns with epitope translation efficiency. Validate epitope hierarchy shifts using OT-I/CD8+ T cell proliferation assays and single-cell TCR sequencing37.

Work Package 3: In Vivo Immunodominance Profiling and Functional Validation
Objective: Evaluate how LNP-mRNA combinations alter epitope dominance in infection and tumour models.
Methodology: Immunize mice with top LNP-mRNA candidates and challenge with *Listeria*-OVA or E.G7-OVA tumors3. Map CD8+ T cell responses to 15 OVA-derived epitopes via tetramer staining and IFN-γ ELISpot. Deplete cDC1/cDC2 subsets to assess their roles in immunodominance using XCR1-DTR mice3.

Training opportunities
This project will elucidate how LNP-mRNA design parameters govern T cell epitope selection, enabling precision engineering of vaccines with tailored immunodominance profiles and will provide training in human primary cell culture, advanced immune profiling techniques including spectral flow cytometry, RNAseq and mass spectrometry as well as molecular and chemical biology techniques.

References
1. Alameh MG, Tombácz I, Bettini E, Lederer K, Sittplangkoon C, Wilmore JR, Gaudette BT, Soliman OY, Pine M, Hicks P, Manzoni TB, Knox JJ, Johnson JL, Laczkó D, Muramatsu H, Davis B, Meng W, Rosenfeld AM, Strohmeier S, Lin PJC, Mui BL, Tam YK, Karikó K, Jacquet A, Krammer F, Bates P, Cancro MP, Weissman D, Luning Prak ET, Allman D, Locci M, Pardi N. Lipid nanoparticles enhance the efficacy of mRNA and protein subunit vaccines by inducing robust T follicular helper cell and humoral responses. Immunity. 2021 Dec 14;54(12):2877-2892.e7. doi: 10.1016/j.immuni.2021.11.001. Epub 2021 Nov 4. Erratum in: Immunity. 2022 Jun 14;55(6):1136-1138. doi: 10.1016/j.immuni.2022.05.007. PMID: 34852217; PMCID: PMC8566475.
2. Zeng Y, Escalona-Rayo O, Knol R, Kros A, Slütter B. Lipid nanoparticle-based mRNA candidates elicit potent T cell responses. Biomater Sci. 2023 Jan 31;11(3):964-974. doi: 10.1039/d2bm01581a. PMID: 36537916

7. Characterising and tracking vaccine specific T Cells in the periphery and in tumour sites, following the administration of novel cancer vaccines in humans – Tim Elliott

Primary Supervisor: Tim Elliott
Additional Supervisors: Ellie Barnes

Abstract of the project
We have used state of the art approaches to characterise the magnitude, phenotype and function of vaccine generated T cells in the blood, after vaccination with ChAdOx1 and mRNA vaccine platforms. These approaches used stimulation of peripheral blood cells with vaccine antigen [1], and more recently cells sorting (using Flow Cytometry) of activated T cells after antigen stimulation (using the AIM assays) for T cell quantification, followed by sc-RNA sequencing of AIM+ T cells for in depth characterisation of T cell phenotype, cell interactions, and TCR repertoire characterisation [manuscripts in preparation/submission]. In this DPhil project you will; (i) apply similar sc-RNA seq approaches to assess in vivo human T cell responses to novel cancer vaccines, (ii) additionally track TCR clonotypes detected in the periphery in response to vaccination, to tissue sites of cancer/pre-cancer and iii) characterise in detail, the immune landscape in patients at high risk of cancer, using multiparametric flow cytometry and correlate this with subsequent vaccine responsiveness (e.g. heavy smokers/lung cancer patients vs healthy controls receiving lung cancer vaccines). This is an exciting opportunity to apply state of the art immune technologies to the development of novel cancer vaccines in humans.

Research objectives and proposed outcomes
Recent advances in cancer vaccines, such as mRNA neoantigen platforms demonstrate the capacity to induce durable, functional CD8+ T cell responses that correlate with prolonged recurrence-free survival in malignancies like pancreatic ductal adenocarcinoma. However, challenges persist in understanding the spatial distribution, clonal persistence, and tissue-specific adaptation of vaccine-induced T cells. Current methodologies combine antigen-specific T cell enrichment (e.g., AIM assays), single-cell RNA sequencing (scRNA-seq) for phenotypic profiling, and TCR clonotype tracking (e.g., CloneTrack) to decode differentiation states and longevity. Despite progress, gaps remain in linking peripheral immunity to intratumoural activity and identifying pre-vaccination immune landscapes that predict clinical efficacy. This project will bridge these gaps using cutting-edge multimodal technologies.

Work Package 1: High-resolution profiling of vaccine-induced T cell responses
Objective: Systematically map the phenotype, transcriptional programs, and clonal dynamics of vaccine-specific T cells in blood using longitudinal scRNA-seq with paired TCR analysis.
Methodology: Enrich antigen-specific CD8+ T cells via AIM assays and flow cytometry; Perform scRNA-seq with integrated TCR sequencing (10x Genomics) to resolve clonotype-specific differentiation trajectories; Apply PhenoTrack

Work Package 2: Spatial mapping of vaccine-specific T cell clonotypes
To link peripheral clonal persistence to intratumoural activity, revealing mechanisms of immune escape or clonal pruning.
Objective: Track peripheral TCR clones to pre-malignant/tumour sites and assess their functional impact on tumour evolution.
Methodology: Cross-reference blood TCR clonotypes with TCR sequences from tumour biopsies using single-cell or spatial transcriptomics; Deploy multiplex immunohistochemistry to spatially resolve clonally **expanded** T

cells in tissue microenvironments; Analyse tumour phylogenetics to evaluate selective pressure on immunogenic neoantigens.

Work Package 3: Immune landscape stratification in high-risk cohorts
Objective: To establish immune stratification frameworks to personalize vaccine timing and combinatorial therapies by defining pre-vaccination immune signatures predictive of response in high-risk populations (e.g., smokers at risk for lung cancer).
Methodology: Use high-parameter flow cytometry (30+ markers to encompass include exhaustion markers, memory subsets, and innate lymphoid populations) to profile baseline immune states in peripheral blood and tissues. Integrate with scRNA-seq datasets from Work Package 1 to identify predictive biomarkers of vaccine responsiveness; Validate findings against reference T cell atlases

Training opportunities
This project will provide training in transcriptomics, spatial biology, and immune monitoring to advance mechanistic understanding and clinical translation of next-generation cancer vaccines.

References
[1] https://www.nature.com/articles/s41591-023-02414-4

8. Deep exploration of the pre-tumour microenvironment in colorectal polyps – Eoghan Mullholland

Primary Supervisor: Eoghan Mullholland
Additional Supervisors: David Church, Simon Leedham

Abstract of the project
Colorectal cancer (CRC) is a highly dynamic and complex ecosystem shaped by the intricate interplay between three distinct tissue compartments – the epithelium, the stroma, and the immune system. These compartments co-evolve, transitioning from the maintenance of intestinal homeostasis to the emergence of precursor lesions, ultimately leading to cancer invasion and dissemination. CRC can take up to 5-17 years to develop from the initial mutational event, providing an exceptional window of opportunity to unravel the pre-cancer biology of CRC and devise novel strategies for cancer prevention.

We have established a cutting-edge analytical pipeline to investigate these pre-cancerous tissues using advanced multi-omic technologies, including whole genome sequencing (WGS), RNA sequencing (RNAseq), single-cell RNA sequencing, organoid models, and spatial transcriptomics. By integrating these approaches, this studentship will aim to answer questions regarding how disruptions in key signalling pathways drive histologically detectable changes, remodel the pre-tumour microenvironment, and shape tissue and cellular interactions across different stages of cancer evolution. We have an extensive bank of Human pre-cancer lesion samples with distinct morphologies, including serrated, conventional, and those derived from familial adenomatous polyposis (FAP).

Research objectives and proposed outcomes
This project will investigate the composition and cellular interactions of the pre-tumour microenvironment in intestinal polyps, aiming to uncover the mechanisms driving pre-cancer development through targeted manipulation of key cellular components in pre-clinical models. Using human tissue samples and state-of-the-art mouse models, we will integrate multi-omic techniques with advanced mathematical analysis to characterise cellular phenotypes within their native tissue context.

Work Package 1: Defining the Pre-Tumour Microenvironment. The pre-tumour microenvironment (pre-TME) consists of stromal cells, immune cells, and matricellular proteins, all surrounding the epithelium. Different mutations give rise to pre-cancers with distinct morphologies, potentially enabling them to remodel their surrounding cellular landscapes and generate diverse pre-TMEs. By applying multi-omic approaches—including RNA sequencing (RNA-seq), single-cell RNA sequencing (scRNA-seq), and whole-genome sequencing (WGS)—to human pre-cancerous lesions, we aim to characterise the composition and nuanced differences across these tissues.

Work Package 2: Spatial Interactions in Colorectal Cancer (CRC) Pre-Cancers. Using advanced multiplex proteomic and spatial transcriptomic technologies, we can map tissue components in situ. This will not only allow us to examine the abundance of different cell phenotypes across tissue regions but also to analyse their spatial interactions. By applying our in-house developed spatial statistics platform, MuSpAn (Bull, 2024), to human pre-cancerous lesions, we aim to identify how cellular interactions vary across different tissue architectures and mutational landscapes.

Work Package 3: Investigating the Impact of Genetic Manipulation on the Pre-TME in Pre-Clinical Models. Using in vivo models of intestinal pre-cancer coupled with in vitro models, we will manipulate key pathways

and assess how different therapies or blockade strategies influence the pre-TME over time. Insights gained from human polyp lesions will guide this work, allowing us to leverage our MuSpAn spatial statistics tool to decipher cellular response signatures—how cell interactions change under different stimuli or inhibition. This will help us to further elucidate the mechanisms underpinning pre-cancer biology in the intestinal setting.

Training opportunities

This project is ideally suited to a candidate eager to engage in multidisciplinary research, gaining expertise in wet-lab techniques, computational analysis, and spatial statistical approaches. With a major research focus in CRC being to decipher the mechanisms driving pre-cancer evolution, this study has the potential to provide invaluable tools and insights into disease progression. The findings could significantly accelerate CRC prevention strategies and contribute to the discovery of novel vaccine targets

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