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Together we will beat cancer

DPhil In Cancer Science University of Oxford

2020 Intake Project Book

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Introduction

This handbook provides an overview for prospective students looking to study for a DPhil in Cancer Science starting in 2020 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 an influential cancer researcher of the future.

Selection Criteria & Eligibility

There are three tracks in the programme as described below, meaning that non-clinicians, undergraduate medical students and post-graduate medical trainees are all eligible to apply for the fully funded (at home rate) studentships.

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

Application Track 2 – Medical Undergraduates. Medical students who are currently undertaking a primary medical qualification (MBBS, MBChB or equivalent)

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

All applicants will be judged on the following:

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

Funding

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

- Application Track 1 – 3 years of salary at Grade E64 Clinical Researcher rate.
- Application Track 2 – 3 years of stipend at the flat rate of £19,000 per annum.
- Application Track 3 – 4 years of stipend at the flat rate of £19,000 per annum.

International applicants are eligible, however funding is limited to the Home level for this programme and therefore international applicants would need to either source further funding or support themselves financially for the remaining fees.

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 January 10th 2020. Shortlisted students will be invited to interview in February. If successful, students will be allocated a project on the basis of their ranking during the review process. It is strongly suggested that students contact supervisors of projects they are interested in applying for prior to application.

Projects at a Glance

Projects are listed below in the following structure "Title – Main Supervisor [Eligible Application Tracks]"

1. Modelling the effect of vascular architecture on the outcome of combination therapies – Prof. Maini[3]

Primary Supervisor: Philip Mani

Additional Supervisors: Joe Pitt-Francis, Ruth Muschel, Helen Byrne

Eligibility: Track 3 students only are eligible to apply for this project.

Required Background: Software developer or mathematical modelling background.

Project Summary

Project Abstract
Two of the main cancer treatments – radiation and stage-dependent chemotherapy – are critically affected by the oxygenation status of a tumour. This, in turn, depends on the architecture and integrity (leakiness) of the vasculature. While the effect of oxygen on these treatments is reasonably well understood, little is known about how different vascular architectures affect oxygen delivery. Furthermore, the vasculature is continually changing due to angiogenesis and the effect of therapies on the endothelial cells. The aim of this research proposal is to develop a computational model to determine how different vascular architectures affect oxygen delivery, which will lead to the first computational study of how anti-angiogenesis and radio/chemotherapy treatment should be combined to maximise tumour cell kill.

Research Objectives and Proposed Outcomes
It is now clear that the relationship between oxygen delivery and tumour blood vessel density, geometry and status is more complex than originally envisioned. Counter-intuitively, anti-angiogenesis treatment has been shown to often increase oxygenation in a clinical setting. It has been proposed that this happens via the phenomenon of "vascular renormalisation", in which previously leaky newly formed vessels become less leaky. Moreover, our own computational studies have shown that, due to intrinsic heterogeneities in tumour properties, reduction in vessel density can have a biphasic effect on oxygen delivery, reducing it for initially low vessel densities (as previously envisioned), but increasing it for initially high, more pathological, vessel densities [2]. Clearly, how oxygen delivery depends on the blood vessel architecture, a question that is of fundamental importance to understanding the dynamics of a tumour, is not understood. Compounding the issue is that oxygen is delivered to the tissue by blood-borne haematocrit and there is no standard model on haematocrit-splitting at vessel junctions. Recently, we have developed a new haematocrit-splitting law [3] and shown how this phenomenon, combined with abnormal vessel geometry of a tumour, may contribute to oxygen heterogeneity within the tumour (Figure 1). In this project, we propose to model the delivery of oxygen to tumours using a computational model and to investigate how delivery depends on (O1) vessel geometry and architecture; (O2) vessel permeability and leakiness; (O3) continuing vessel remodelling due to angiogenesis and vessel pruning. We will then use the computational model to investigate how the predicted oxygen profiles affect the efficiency of radio/chemotherapy, bearing in mind that these therapies also affect the endothelial cells. We will also consider these therapies in combination with anti-angiogenesis treatment to identify those strategies that maximise tumour cell kill.
This is a multidisciplinary proposal involving 4 co-applicants: Philip Maini and Helen Byrne (Mathematical Institute), who developed the first multiscale computational models for tumour angiogenesis that included feedback between tumour hypoxia and vessel remodelling [2] and have extended these studies to include finer biochemical detail [4]. They are now working with Joe Pitt-Francis (Computer Science) who played a key role in developing MC, and Ruth Muschel (Oncology) [5], whose group has provided experimental images of vessel architecture that can be loaded into MC, and who has data on how anti-angiogenesis treatments affect vessel architecture. Together, this group, through funding from other bodies (EU 7th Framework, Marie Curie, CRUK Oxford Centre) has developed the basic infrastructure needed to be the first to address the above questions. The award of a studentship will enable them to exploit the work they have already started, to produce high

impact research that will establish them as the only group using experimental data to develop predictive models for different combination treatments.

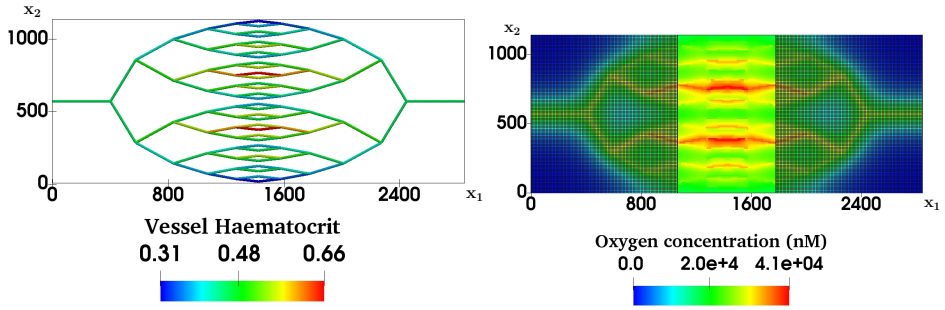

Figure 1: Preliminary computational modelling using Microvessel Chaste indicates that haematocrit distribution within a forking network of 6 generations of vessels (left) and the tissue oxygen distribution (right) are highly heterogeneous when haematocrit splitting depends on the distance between vessel branch points.

Translation

Much research in tumour angiogenesis and radio/chemotherapy has been based on investigations of key processes in isolation. This is a necessary first step. The next step is to integrate these findings into a fully multiscale model; only then can the effects of the interactions between the processes be fully understood. By validating our model predictions against experimental data, we will be able to use imaging data on tumour blood vessel architecture to determine how best to apply certain combination therapies to tumours.

References

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[2] Alarcon, **Byrne**, **Maini**, A cellular automaton model for tumour growth in inhomogeneous environment *J.Theor.Biol.*, 225, 257-274 (2003)

[3] Bernabeu …, **Maini**, **Pitt-Francis**, **Muschel**, …, **Byrne**, Abnormal morphology biases haematocrit distribution in tumour vasculature and contributes to heterogeneity in tissue oxygenation (to be submitted *PNAS*)

[4] Connor, … **Maini**, **Byrne**, An integrated approach to quantitative modelling in angiogenesis research*, J.R. Soc. Interface*, 12, 20150546 (2015)

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2. Interrogating the myeloma-bone environment using microfluidics – Prof. Edwards[1,2,3]

Primary Supervisor: Claire Edwards

Additional Supervisors: Srinivasa Rao, James Edwards, Edmond Walsh

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: Either a biomedical/biological/biochemistry or an engineering background.

Project Summary

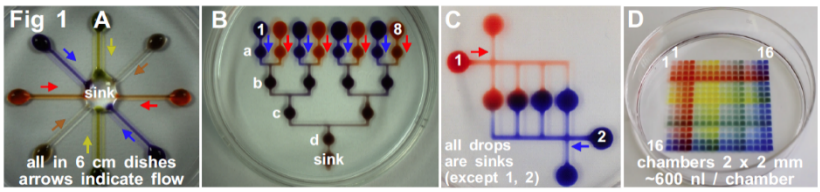
Abstract
Once tumours develop within bone they are largely incurable, and modelling the complex cellular interactions within this specialised environment is essential for the development of new therapies. This project brings together the unique expertise of Profs Edwards and Walsh in bone oncology and engineering/microfluidics; we plan to develop microfluidic devices facilitating study of interactions between the many cell types involved in multiple myeloma including osteo-blasts/-clasts, bone marrow stromal cells (BMSCs), adipocytes, plus immune and endothelial cells. We will analyse the myeloma-bone transcriptome, and identify key cellular/molecular interactions driving disease progression. We hope to uncover new therapeutic targets for the treatment of this fatal malignancy.

Research Objectives
Academic value of research: Cell interactions in the bone microenvironment are integral to tumour growth and survival in myeloma, and there is a need to effectively recreate them in vitro. Current approaches are limited to culturing one or two cell types, so most interactions underpinning disease progression and therapeutic response are missed. Our goal is to develop a tumour microenvironment replicating that found in vivo, using myeloma as an exemplar.

Aims/Objectives:
1. Develop microfluidic devices mimicking the cellular interactions found in the myeloma bone marrow.
2. Generate transcriptomes during premalignant (MGUS), early-, and late-stage disease.

Background and Preliminary Studies
Multiple Myeloma: Myeloma is a fatal haematological malignancy exquisitely dependent on cell interactions in the BM. Using *in vivo* murine models, we recently performed transcriptome profiling of cells in the myeloma-bone niche, identifying BMP signalling in osteoprogenitors as a new therapeutic target (Gooding *et al.*, *Nat. Comms*)(1). This highlights the translational impact that can arise from such profiling, and points to the need for *in vitro* replicates of such microenvironments.
Microfluidics: Incorporation of microfluidic devices into oncology is an emerging area with potential for significant impact in basic and translational studies. We have developed novel microfluidic technologies that exploit unique properties of fluid walls built by reshaping two liquids – cell-growth media and the immiscible fluorocarbon, FC40 – sitting on standard Petri dishes (**Figure 1)**(2, 3). Different cell types (sometimes from biopsies) will be grown in micro-chambers; each is completely isolated from others by immiscible fluid walls only a few tens of microns thick that prevent all exchange of reagents (just like solid walls). As these walls can be made, destroyed, and reconfigured on demand around living cells(4), we can allow/prevent diffusional or active flow/advection between different cell types at will, and monitor effects on phenotype (e.g., transcriptomes using single-cell RNA-Seq). We can also select cells of particular interest in heterogenous populations (e.g., dormant or drug-resistant/responsive tumour cells), build isolating walls around them, and then analyse phenotypes.

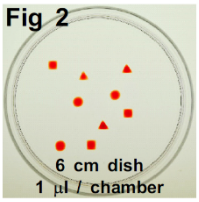
Using this approach, we propose to recreate the symbiotic cellular cross-talk between multiple cell types within the tumour-bone microenvironment to create a strong platform for drug screening and mechanistic studies.



PLAN OF WORK

Aim 1: Develop microfluidic devices to mimic cellular interactions found in myeloma bone marrow (BM), through recapitulation of cell interactions. Microfluidic devices will be designed in a step-wise fashion, starting with one just for myeloma plus BMSCs, and building up to a multi-cell system (myeloma cells, BMSCs, osteo-blasts/-clasts, endothelial/immune cells, adipocytes). Flow between distinct cell populations will be controlled by Laplace pressure, and 'valves' closed/opened using a fluoro-/hydro-philic 'stylus' to facilitate cell migration. Cell number, culture conditions and plating density will be optimised for each cell type, with expertise in bone cell culture provided by Prof. James Edwards, NDORMS. Microfluidic devices will be combined with high-throughput imaging for longitudinal monitoring. Functional endpoints within the microenvironment populations include differentiation, mineralisation, and formation of lipid droplets. Biological endpoints within myeloma cells will include cell number, proliferation, apoptosis, and migration – initially using cell lines, with subsequent progression to primary MM cells and non-tumour bone cells from the BM of MM patients.

Aim 2: Model myeloma-bone microenvironment at distinct disease stages. Cellular changes occurring during myeloma are well described but genetic ones in the tumour-bone microenvironment are poorly understood. We will model distinct stages of myeloma pathogenesis within devices using premalignant/MGUS (BM cells isolated from a murine model of MGUS), early disease (myeloma cells, limited bone disease reflected by elevated but normal osteoblasts), and late-stage disease (drug-resistant myeloma cells and extensive bone disease represented by elevated osteoblasts and reduced osteoblasts). RNA-Seq will be performed on individual cell populations to elucidate genetic changes occurring within distinct tumour/BM cells during disease progression (in collaboration with Dr. Rao, NDS). We will exploit the excellent optical clarity provided by fluid walls. Thus, the various cell types adopt complex morphologies *in vitro*, with some types congregating with myeloma cells; then, we will isolate congregates of interest by 'printing' fluid walls around them (illustrated in **Fig. 2** at the macroscale, where circles, triangles, or squares were printed around selected cell clusters), prior to cell recovery and RNA-seq. Results of transcriptomic analysis will be validated using in situ immunofluorescence and functional studies.



EXPECTED OUTCOMES:

We anticipate the following results**:** (i) Microfluidic devices will be developed facilitating the culture of 2-6 cell types, with optimised culture conditions and functional outcomes; flows within these devices will be characterized. (ii) These devices will enable analysis of the intercellular communication occurring between primary myeloma cells and the BM microenvironment (e.g., which cells promote/limit tumour growth?). (iii) Transcriptomic profiles will be generated for myeloma cells and distinct cell populations from the BM microenvironment during myeloma pathogenesis.

Significance and impact of the project

Tumour cells are usually studied *in vitro* in isolation, free of any relevant microenvironment (e.g., drug screens may include tumour cells, but generally not neighbouring ones found *in vivo*). We hope our development of a simple microfluidic approach – one accessible to biologists – will establish a paradigm for the study *in vitro* of many tumours in a more natural microenvironment than hitherto, and that this will find wide application in oncology since the approach can easily be extended to other tumours metastasising to bone. We anticipate that inclusion of the microenvironment will improve drug screening, and drive identification of novel targets that have a greater likelihood of being effective in the clinic. Moreover, the microfluidic approaches developed here will be extremely well-suited to the analysis of patient samples which inevitably contain only a few cells.

References

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3. Single-cell analysis of haematopoietic stem cells in *SF3B1* mutant MDS: identification of new therapeutic targets/treatments – Dr. Pellagatti[1,2,3]

Primary Supervisor: Andrea Pellagatti

Additional Supervisors: Adam Mead, Supat Thongjuea

Collaborator: Amit Verma

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: A biomedical/biological/genetics background.

Project Summary

Abstract

The myelodysplastic syndromes (MDS) are common myeloid malignancies.[1] There are few effective treatments for MDS and the vast majority of patients will die as a result of their disease. The MDS originate in bone marrow haematopoietic stem cells (HSCs) that are essential for disease initiation and progression, and the eradication of the malignant HSCs is necessary to achieve cure. Splicing factor mutations are the most common mutations found in MDS. The splicing factor gene *SF3B1* is the most frequently mutated gene in MDS, and results in aberrant pre-mRNA splicing.[1-3] *SF3B1* mutations have been identified in other cancers, including chronic lymphocytic leukemia, uveal melanoma, breast cancer and pancreatic cancer, suggesting that somatic mutations in spliceosome genes have an important role in tumorigenesis.[1] We will use a novel single-cell method for high-sensitivity mutation detection with parallel RNA sequencing analysis (TARGET-seq)[4] to determine the genetic and transcriptomic heterogeneity of single HSCs from individual MDS patients harbouring *SF3B1* mutations. Our approach allows the detection of changes in gene expression levels and also aberrantly spliced transcripts. Dysregulated genes/pathways will be identified using appropriate bioinformatics analyses[5] in mutation-defined HSC subpopulations in each MDS patient (Figure 1) and used as input to identify drugs that can target the different HSC subpopulations in an individual patient. The identified drugs will be tested *in vitro* using long-term culture-initiating cell (LTC-IC) assays to determine the drugs that are cytotoxic to MDS HSCs (with the relevant mutation profile) while sparing the HSCs from healthy controls. Importantly, the drugs identified may also have efficacy in other myeloid malignancies with similar mutation profiles. This approach will lead to the identification of new treatments for *SF3B1* mutant MDS and has broad applicability to other haematological malignancies and other cancers.

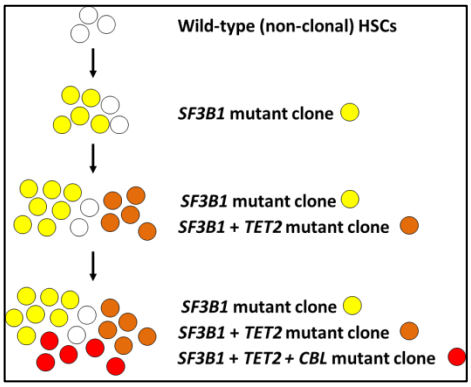

Figure 1. Sequential mutation acquisition in MDS. An example of clonal hierarchy in an MDS patient harbouring *SF3B1, TET2* and *CBL* mutations is shown. Using TARGET-seq, we will profile the single-cell transcriptome for individual MDS patients and identify different HSC subpopulations on the basis of their mutation status. In a case

with *SF3B1* as a founder mutation, with *TET2* as a subsequent mutation, and *CBL* as a late mutation, the HSC subpopulations could comprise cells with *SF3B1* mutation only, cells with both *SF3B1* and *TET2* mutations, cells with all three mutations, as well as wildtype cells (non-clonal).

Research objectives and proposed outcomes

The aim of this project is to identify new therapeutic targets/treatments from the transcriptomic analysis of single HSCs (i.e. the disease-propagating cell population in MDS) from MDS cases with *SF3B1* mutation. Our objectives are:

1- To perform single-cell mutational analysis with parallel transcriptomic (RNA-seq) analysis on the HSCs of a group of MDS patients harbouring *SF3B1* gene mutations

2- To perform bioinformatics analysis on the single-cell data in order to identify molecular signatures and druggable targets, and drugs known to target them, in each mutation-defined HSC subpopulation in the MDS patients

3- To test the identified drugs in vitro to determine the drugs which are cytotoxic to MDS HSCs (with the relevant mutation profile) while sparing the HSCs from healthy controls

We will identify dysregulated genes (both at the expression and splicing levels) and pathways in the HSCs of *SF3B1* mutant MDS patients, with the potential to discover of novel pathways involved in MDS pathogenesis. This will broaden our knowledge of the MDS HSC pathophysiology.

We will identify drugs and drug combinations that can specifically target all HSC subsets within each MDS patient, enabling treatment tailored both to the individual MDS patient (precision medicine), as well as to whole groups of patients with *SF3B1* mutations and mutation combinations. Toxicology and safety data may be already available for some of the drugs identified and this will accelerate their introduction into the clinic to treat MDS as a new disease indication for these drugs. This project will benefit from collaboration with Professor Amit Verma (New York), an expert in MDS HSC biology and drug target identification from gene expression data in myeloid malignancy.

Translational potential

We will identify new drugs and drug combinations for MDS patients with *SF3B1* mutations. The drugs identified may also have efficacy in other myeloid malignancies (e.g. acute myeloid leukaemia) and other cancers with *SF3B1* mutations. This approach will lead to the identification of new treatments for *SF3B1* mutant MDS and has broad applicability to other haematological malignancies and other cancers.

References

1. Pellagatti A, Boultwood J. Splicing factor gene mutations in the myelodysplastic syndromes: impact on disease phenotype and therapeutic applications. *Adv Biol Regul* 2017; 63:59-70.

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4. Developing a facile on chip p53 autoantibody electroanalytical assay platform -Prof. Davis[1,2,3]

Primary Supervisor: Jason Davis

Additional Supervisors: Xin Lu

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: A biomedical/biological/chemistry background.

Project Summary

Abstract
Early detection has the potential to transform cancer care by preventing tumours from acquiring the characteristics of advanced malignancies. This opens the prospect of preventing malignant cancer, minimising therapeutic toxicity arising from treating established tumours and reducing the economic burden of advanced cancer. Conventional screening is known to reduce mortality, but uptake is poor in many populations. The most economically viable and scale-able means of cancer screening is through the use of a non-invasive "liquid biopsy" where a highly sensitive detection format can be applied to the robust quantification of key markers within a few microlitres (literally a droplet) of blood. The tumour suppressor, p53 is the most mutated gene in human cancer. When mutated, p53 often mis-folds and aggregates, inducing the production of p53 autoantibodies (p53-AAbs). These have been detected in 18 out of 20 cancer types tested, with especially high levels in gastrointestinal cancers. Despite the ability of p53-AAbs to be detected in high-risk cancer populations even years before cancer diagnosis, clinical usage has been hampered by the low sensitivity of current detection methods, such as ELISA. *The aim of this project is to build upon substantial preliminary work in establishing a robust, on chip, means of quantifying p53 autoantibodies from a small droplet of blood within a few minutes at very low cost and high sensitivity.* The vision is that this would underpin the development of a prognostic liquid biopsy that could be applied at scale.

Research objectives and proposed outcomes
Patients with a "p53 mutation"/p53 protein overexpression have a markedly poorer prognosis in esophageal and colorectal cancers.[1, 2] p53 mutation and overexpression induces p53 autoantibodies that can be assayed through the use of surfaces expressing specific antigen epitopes. Although p53-AAbs have been detected with 95% selectively for mutant p53, to date these are only detectable in ~30% patients with mutant p53 – a significant current limitation is the low detection sensitivity of current technology (ELISA) and the use of unmodified short (low affinity) peptide recognition sequences.

Development and optimisation of an electrochemical marker detection platform
One of the most profound challenges associated with measurements in biological fluids is a common inability to capture specific target molecules in a massive excess of chemically complex background. This is the first challenge. Current protein and antibody-based assays are typically laborious, slow, require a specifically-labelled secondary antibody for every antigen of interest and struggle to reach limits of detection relevant to many cancers. This is the second challenge. Electrical Detection Assays, can be exceedingly sensitive and high throughput and are generated by controllably immobilizing receptive biomolecules on electrodes and converting the target protein binding event into a measureable electrical signal. We have shown that electrode surfaces can be controllably modified such that very specific marker detection can be carried out in blood with exceedingly low detection limits in a single step.[3, 4 5] The analytical (impedance and capacitance) methods are potentially very low cost, and readily multiplexable (herein potentially integrating multiple peptide sequences as baits). These methods routinely out-perform standard ELISA or chemiluminescence assays in terms of sensitivity,[5] user intervention/error and time, and we have also shown that antigen-expressing surfaces can be used to support the assaying of autoantibodies.[6, 7] Herein we will also utilise full length post translationally modified peptides as baits (these more completely reflect the physiological epitopes).

Optimising assay dynamic range

The hyperbolic dose-response curves associated with an interfacial recognition event can be limiting in terms of acquiring an ability to respond adequately to an analyte over a sufficiently broad concentration range. By using sets of different receptors, nature has evolved so as to not be limited in this way. We will explore and then utilise exactly this concept herein once binding characteristics have been mapped across electrode arrays using variable peptide bait surface densities (and thus sensitivity and response saturation profiles); individually addressed electrodes will present a unique detection limit and dynamic range that will be a reflection of its receptive layer composition. In analysing the mean response of the combined electrodes a weighted mean of these is presented with an increased dynamic range. **Kinetic Quantification**. The interfacial binding of an analyte can be considered as the sum of two sequential processes, (diffusion from bulk to interface across the diffusion layer and then reversible binding at the interface). At an effective capture interface (see above) target binding will be largely mass transport limited and the time-dependent sensor response dependent on active analyte concentration, its diffusion coefficient, flow conditions and dimensions of the cell. This approach will be built upon in this project to enable a new form of target marker quantification.

Specific objectives

1. To develop new on-chip peptide-supporting high-surface area polymer films supporting highly selective p53 Ab recruitment (initially using commercial monoclonal Ab).
2. To develop and utilise new label free molecular detection protocols based on antibody capacitor films.
3. To increase marker detection dynamic range using arrays.
4. To establish a biomarker quantification protocol based on time dependent signal change (kinetic quantification)
5. To further validate the specificity of these markers in other cancers.
6. To correlate EIS derived sensitivities and specificities with those obtained through conventional ELISA methods using the same peptide baits.

References

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5. Development of a single cell sequencing and computational biology platform to evaluate the interaction between the microenvironment, tumour metabolism, and immunity – Dr. Lord[1,2,3]

Primary Supervisor: Simon Lord

Additional Supervisors: Francesca Buffa, Kim Midwood, Mario Buono, Gillian Farnie, Adrian Harris, Skirmantas Kriaucionis

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: A biological, biomedical or engineering background.

Project Summary

Abstract
The tumour microenvironment plays a key role in cancer progression and in mediating response to many therapies. Single cell functional genomic profiling is already delivering on its potential to provide insight into these complex relationships. However, single cell sequencing snapshots, which have been the only clinically viable option, are not sufficient to provide the needed high-resolution to resolve what it is a multi-layer fast-evolving dynamic system. We propose to establish a platform for the single cell sequencing of solid tumour samples combining transcriptomic and DNA methylation profiling of multiple tumour cell compartments. This project will draw on cross-departmental expertise in evaluating the interaction between tumour metabolism, the microenvironment, immunity and, **notably, the extracellular matrix,** for solid tumours. We will develop machine learning and mathematical modelling to complement standard integrative genomic approaches to interrogate these interactions with cross-project applicability. Several different projects, led by separate PIs, with ethical approval already in place and matched funding, will provide data. These projects will address a spectrum of research questions that will act as the ideal testbed to optimise both experimental protocols and computational biology techniques.

Research objectives/proposed outcomes

1) Establish a platform for standardised handling of solid tumour samples, cell sorting and transcriptomic/DNA methylation single cell sequencing
2) Develop computational biology approaches using machine learning and mathematical modelling techniques for high throughput analysis

Work packages

1) Pilot work using FACS sorting of human breast tumour alone to optimise amount of tissue required to characterise all cellular compartments of interest (led by Mario Buono)
2) Pilot work to optimise preparation of samples for single cell DNA methylation sequencing (sample digestion, FACS sorting) - (led by Skirmantas Krauicionis)
3) Use sequencing data and individual project objectives (**see below**) as a compass to develop computational biology approaches that can characterise the relationship between tumor cells and other cells in the tumour microenvironment in a manner that is reproducible and has potential to be high throughput

Development of computational biology techniques to dissect the relationship between immunity, the tumor microenvironment, and tumor cell phenotype – Buffa group
This project will be developed by a non-clinical bioinformatic DPhil student supervised by Francesca Buffa and is the driver for the establishment of a tailored Computational Biology infrastructure. The Buffa's lab work focuses on using integrative genomic techniques, machine learning and computer simulations to understand how the

microenvironment interacts with tumor cells. To date this research has provided new insight into the function of several previously uncharacterized genes and generated robust gene signatures which are being evaluated as biomarkers to be translated to the clinical setting. A limitation has been that most of the current genomic profiling studies are based on mixed tumor-stroma samples using bulk sequencing and single cell sequencing will alternatively allow quantification of intra-tumor heterogeneity, to reveal subtle, but biologically important, patterns. Prof Buffa has recently been awarded a European Research Council Programme to develop computer simulations of cancer cell behaviour aimed at predicting cancer occurrence and personalising cancer treatment. The DPhil student will work in close collaboration with this programme and would benefit from access to the necessary computing tools, and help from one of the post-doctoral scientists.

Interactions between the tumor matrix and immune cell behaviour – Midwood group

Our hypothesis is that whilst inflammatory signals from host-derived extracellular matrix are key components of immune defence against cancer, tumors exploit the matrix to evade immune surveillance, creating a tolerogenic microenvironment by restricting immune cell infiltration/positioning or directly signalling to repolarize myeloid cells and promote lymphocyte anergy. Targeting selected tumor-derived matrix molecules is effective in preventing tumor growth and spread, however the complex crosstalk between the matrix, tumor, stromal and immune cells is not yet well characterized. Single cell RNA seq will be used to assign matrix gene expression signatures to tumor and host (stromal and immune) cell subsets, to interrogate the hypothesis that population specific matrix networks are better biomarkers of disease prognosis than global signatures derived from bulk data. We will also refine immune cell clusters at a higher resolution before analysis of reciprocal relationships between these populations with tumor and stromal cells from the same patient. Using these data as a blueprint to design novel imaging panels of population specific markers we will map the anatomic location, and interaction networks, of cell subsets within the context of their local matrix. This will provide new insight into pathogenic cell-matrix circuits that can be targeted to re-educate the immune status of the tumor microenvironment.

Development of single cell DNA methylation profiling – Kriaucionis

Single-cell epigenetic profiling is far less utilized than single-cell transcriptomics because the current methods (bisulfite sequencing) for detecting epigenetic modifications are not feasible for small or rare cellular subpopulations. Skirmantas Kriaucionis is currently working with Chunxiao Song at the Ludwig Institute to develop novel bisulfite-free and base-resolution sequencing technologies for DNA methylation and hydroxymethylation. Work is ongoing to assess the technique using pre-clinical models but within 6 months profiling of human tumor samples should be viable.

Mechanisms of endothelial differentiation to normalise tumor vasculature – Harris/Buono

Our hypothesis is that aberrant differentiation of tumour endothelial cells (ECs) causes abnormalities in tumour blood vessels and rectifying these abnormalities is a more effective approach to target tumour angiogenesis than standard approaches. In conjunction with work using in vitro EC model systems single cell sequencing will assess the extent of heterogeneity in normal and tumour EC populations to (i) elaborate differences between normal breast and tumour ECs, including cell surface proteins; (ii) map molecular differentiation stages in primary normal and tumour ECs onto stages identified in the in vitro work outlined above in order to define potential differentiation blocks in tumour ECs; and (iii) identify potential molecular targets that could normalise tumour ECs.

Translational potential of project

Clinical data and blood samples will be routinely collected to match circulating markers and measures of patient metabolism to the tumor sequencing signature (REC approval in place for this). All sequencing and clinical data will be held within the 'dataset manager', cBioPortal, with a view to analysis by other groups across Oxford and in time as an open access resource (database set up for related tissue collection project has already been actioned). Medium-term goals will include the identification of novel drug targets and mechanisms of resistance to therapy. Long-term it is envisaged that the platform will allow for assaying of clinical trial samples to characterise microenvironmental and tumour pharmacodynamic responses to drug therapy.

6. Targeting DNA repair mechanisms in precision cancer therapies – Prof. Lakin[1,2,3]

Primary Supervisor: Nick Lakin

Collaborators: T.Milne, D. Ebner, S. Mohammed.

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: Either a biomedical/biological or physical sciences background.

Project Summary

Abstract
DNA in our genomes is under assault from a variety of agents that induce DNA damage and repair of these lesions underpins human health. Recently, small molecule inhibitors of DNA repair mechanisms have emerged as key agents in cancer therapy, either in monotherapies that exploit the genetic makeup of cancer cells to specifically sensitise tumours to these agents, or in combination therapies that increase the therapeutic index of existing chemo- and radiotherapies[1,2]. Principal in this strategy is inhibition of Poly(ADP-ribose)-polymerases (PARPs), a family of enzymes that regulate repair of DNA breaks, and PARP inhibitors are currently being used to treat tumours with defects in homologous recombination DNA repair. However, whilst this approach is successfully treating breast and ovarian tumours[3], there is a need to broaden the use of PARP inhibitors to treat other cancers.

Building on our extensive expertise in PARP biology and DNA repair[4-8], the overall goal of this research is to address this fundamentally important question by characterising novel cancer-related genes whose disruption is toxic in combination with PARP dysfunction, a concept know as synthetic lethality. We will drive this transformative understanding through multidisciplinary hypothesis-driven research that exploits cutting edge genome editing, proteomics and cell biology to characterise novel genes that are synthetic lethal with PARP dysfunction. This will provide unprecedented insights into how cells maintain genome integrity and novel strategies that target these pathways in precision cancer therapies.

Research Objectives
To identify genes that are synthetic lethal with PARP dysfunction, we performed a CRISPR-based screen of 19,050 genes to identify genes whose disruption specifically kill cells with deleted PARP1 and PARP2 genes (*parp1/2Δ* cells). In addition to gene disruptions previously known to be toxic in combination with PARP inhibitors, we identified a novel gene (*PARP and ATR Synthetic Lethal 19; PASL19*) that is synthetic lethal with both PARP dysfunction and inhibition of the cell cycle checkpoint kinase ATR. Independent disruption of the *PASL19* gene sensitises cells to DNA damage, indicating a role for this gene in the DNA damage response. The overall goal of this research is to exploit a variety of genetic, cell biology and biochemical approaches to define the role of PASL19 in the DNA damage response and cancer therapy by:

a) Identifying which DNA repair mechanism PASL19 regulates
b) Defining the mechanistic basis of this regulation
c) Establishing the relationship between PASL19 and PARPs in the DNA damage response
d) Characterising cancers with defects in PASL19 and the ability of PARP inhibitors to treat these tumours.

Translational Potential:
Currently the use of PARP inhibitors is restricted to treating breast and ovarian tumours with defects in homologous recombination. Defining the role of PASL19 and other genes that are synthetic lethal with PARP inhibitors we will identify novel strategies to broaden the use of PARP inhibitors to treat additional malignancies.

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7. Unravelling the Crosstalk between Immune Signalling and Exosome Biology in Colorectal Cancer – Prof. Powrie[1,2,3]

Primary Supervisor: Fiona Powrie

Additional Supervisors: Deborah C I Goberdhan

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: A biomedical/biological background.

Project Summary

Project Abstract

Colorectal cancer (CRC) is the third most common malignancy worldwide and the fourth leading cause of cancer-related deaths. Current therapy for CRC entails surgical resection of the tumour and adjuvant chemotherapy, to which tumours respond variably. For example, worse prognosis and poor treatment responsiveness is observed for the 45% of CRC tumours that harness a mutation in the gene *KRAS*, locking cells in an active state which drives sustained cell proliferation. While anti-tumour immunity, including IFNγ, is generally associated with favourable prognosis, the Powrie lab and others have identified detrimental roles for IL-23/Th17-driven inflammation in CRC tumours, illustrated in **Figure** [**1, 2**]. Indeed, inhibiting IL-23 or the downstream cytokine IL-22 reduces tumour burden in murine models of both sporadic and colitis-associated CRC [**2**-**5**]. Using a training and validation transcriptomic data set, the Powrie lab has found that *KRAS* mutations are associated with significantly reduced relapse-free survival in stage II/III colon cancer patients when RNA expression of *IL22RA1* was high, but not when it was low (submitted; [**6**]); the same association was observed in patients with metastatic CRC.

Meanwhile, the Goberdhan group has found that when the *KRAS* mutant HCT116 CRC cell line is subjected to nutrient stress by glutamine depletion, it switches to secretion of a subtype of amphiregulin (AREG; EGF ligand)-containing exosomes. These nano-sized vesicles are formed in previously unrecognised exosome-generating endosomes. These compartments and the exosomes that they produce are labelled by the recycling endosomal protein, Rab11a. Rab11a-exosomes modulate glutamine metabolism, promote the growth of CRC cells under reduced serum conditions and new blood vessel formation *in vitro* and *in vivo*, as shown in **Figure** [**7**]. Extracellular vesicles are known to affect the immune system [**8**], and have recently been show to express PD-L1 which can suppress anti-tumour immune responses [**9**]. However, the effects of different CRC exosome subtypes on the anti- and pro-tumorigenic arms of immune signalling have yet to be tested, e.g. could the Rab11a-subtype's growth factor-mediated or metabolism-modulating (cf [**10**]) activities affect specific immune cells and/or cytokine secretion?

The Powrie and Goberdhan groups will initiate a collaboration to investigate the interplay between immune and CRC exosome signalling, testing whether IL-22 modulates exosome secretion from CRC cells harbouring different mutations and whether CRC exosome subtypes have discrete effects on immune cell signalling.

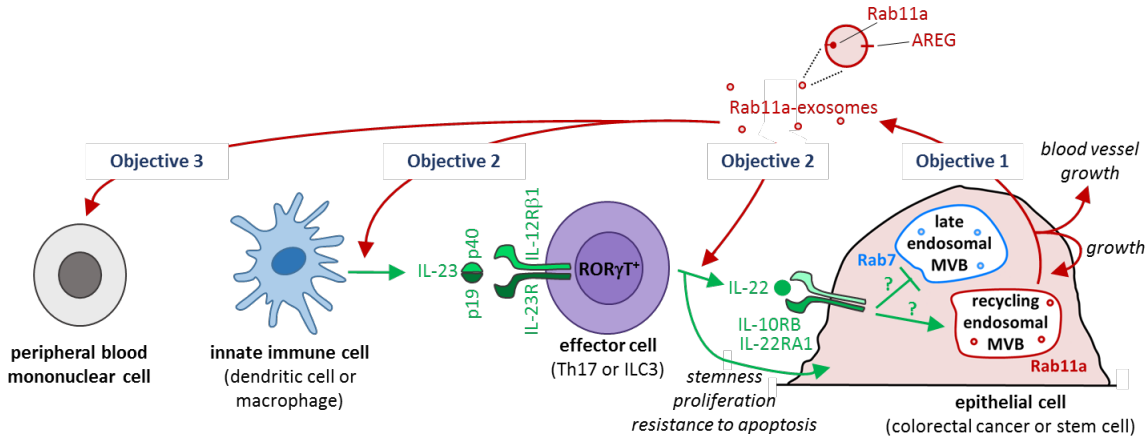

Figure. This proposal will examine *firstly*, how the immune system modulates CRC exosome signalling (Objective 1) and *secondly*, how CRC exosome signalling (illustrated here by nutrient-regulated Rab11a-subtype secretion) affects pro- and anti-tumorigenic signalling by innate immune cells (Objective 2) and PBMCs (Objective 3). MVB denotes a multivesicular body; AREG (Amphiregulin) is an EGFR ligand; IL denotes interleukin.

Research Objectives and Proposed Outcomes

Our research objectives are:

1. To test whether candidate cytokines (eg IL-22, lymphotoxin) modulate secretion of exosomes and other extracellular vesicles (EV) from CRC cell lines, including a KRAS wild-type and mutant isogenic line.

   Outcome: determine whether pro-tumorigenic immune signalling mediates any of its effects via changes in EV and exosome secretion, and how specified tumour-driving mutations impact on this.

2. To test whether CRC EVs produced under different nutrient stresses stimulate production of pro-tumorigenic mediators like IL-6 and AREG, generating cross-talk between tumour and immune cells.

   Outcome: determine if specific CRC exosome subtypes modulate the pro-tumorigenic immune response to stimulate tumour growth

3. To screen for effects of CRC EVs on other immune cells, using firstly peripheral blood mononuclear cells (PBMCs) from healthy and CRC patients.

   Outcome: assess the impact of CRC exosome signalling on other important immune.

Inflammatory signalling and exosome biology are expanding areas in cancer research, but the mechanistic details underlying their pro-tumorigenic effects and in particular how they may work together are poorly understood. There is significant potential in analysing this interplay in colorectal cancer. Our studies have shown that tumour cells make different exosomes in response to microenvironmental changes during tumour growth and that immune cells secrete specific cytokines to enhance such growth. Moreover, the IL-23 and downstream IL-22 pathway is known to be driven by microbial triggers [**11**]. Understanding the links between these processes may help to explain why inflammation plays such an important role in CRC, making this proposal an excellent fit with the studentship call and areas of strength in the Cancer Centre.

Translational Potential of the Project

The most immediate relevance of the project to cancer treatment in the clinic is the potential of a link between cytokine and exosome signalling, which might allow circulating EVs to be used as multifactorial CRC biomarkers of immune and tumour cell interaction. If this connection is established, blood samples pre- and post-treatment could be provided from a planned Powrie lab, Phase 0 trial to inhibit IL-23 in CRC patients. In addition, our findings could impact on the development of therapeutic avenues for patients with KRAS mutant tumours, which are resistant to currently employed EGFR antibodies.

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8. Early cancer detection from epigenetic changes in cell-free DNA – Dr. Schuster-Böckler[1,2,3]

Primary Supervisor: Benjamin Schuster-Böckler

Additional Supervisors: Skirmantas Kriaucionis, Chunxiao Song, Xin Lu

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: Either a biomedical/biological, computer science, mathematical or engineering background.

Project Summary

Abstract
Cell-free DNA (cfDNA), extracted from patient blood, is very promising biomarker because it could detect not just one but many different types of cancer. The technical challenge is to detect a cancer-relevant signal amongst the large quantities of unrelated DNA fragments from other dying cells that release DNA into the blood stream[1]. Previous research has focused on detecting cancer-specific mutations in cfDNA, but these approaches often fail due to the heterogeneity of mutations in different patients, and the difficulty to detect single base changes from the low quantity of tumour-derived cfDNA.
To overcome these problems, we propose to additionally consider changes in epigenetic marks, occurring at multiple locations in the genome, as a cancer biomarker. This is now becoming possible thanks to a new sequencing method developed by the lab of Dr Chunxiao Song at the Ludwig Institute[2]. TET-Assisted Pyridine-borane Sequencing (TAPS) represents a technological leap that reduces the cost of measuring DNA modifications at base resolution, while simultaneously increasing the yield from small sample volumes. Crucially, TAPS also retains information about genomic mutations in the sample, making it possible to measure genetic and epigenetic changes in one single experiment. By detecting methylation events in entire regions, rather than just mutations at single loci, this approach greatly increases sensitivity. Prof. Kriaucionis and Prof. Lu have recently generated high-resolution maps of methylation in different parts of the upper-GI tract, including Barrett's Oesophagus (BO), a lesion of the lower oesophagus that increases cancer risk 20-fold. Computational integration of these data with public methylation data sets from other tissue types has revealed regions that are uniquely methylated in BO, and in oesophageal adenocarcinoma (OAC). CRUK considers OAC a cancer of unmet need, due to its poor prognosis and the increasing incidence of the disease in the western world[3]. This, and the availability of tissue and blood samples from BO and OAC patients from Prof. Lu's group, make BO and OAC an ideal test case for optimising and testing the assay we propose.
Dr. Song and Dr. Schuster-Böckler have performed pilot experiments applying TAPS to whole-genome sequencing from cfDNA in patients with and without BO. The results suggest that cancer-specific hypermethylated regions

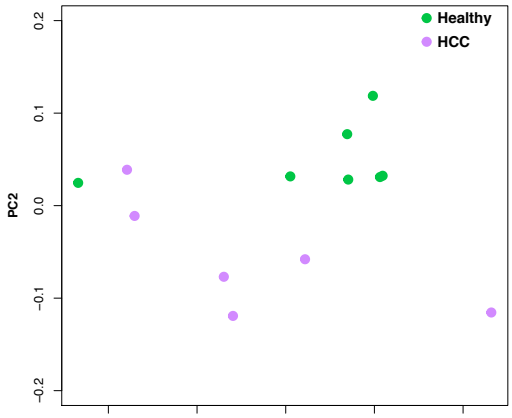

Figure 1 Methylation maps can distinguish liver cancer patients from healthy patients. In this figure, we used TAPS cfDNA data from a preliminary cohort of 6 patients with hepatocellular carcinoma, and 7 healthy controls, to demonstrate that methylation patterns can be used for cancer detection.

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can be detected in blood (Fig. 1). To reduce cost and increase sensitivity, we now plan to create an amplicon-based assay, instead of whole-genome sequencing. In the first instance, we will focus on BO and OAC, but the tools we are developing will be easily transferable to other cancer types.
The work proposed here will establish the utility of targeted epigenetic sequencing for cancer diagnosis. Ultimately, the platform we are developing will enable other researchers in Oxford to convert their unique understanding of epigenetic changes in different tumour types into diagnostic or prognostic tests.

Research objectives and proposed outcomes
Objective 1: Adapt the TAPS technique to be compatible with amplicon-based targeted sequencing
Proposed Outcome: A detailed protocol on how to optimally perform targeted TAPS sequencing
Performed by: Song lab
Objective 2: Design a custom TAPS amplicon panel for BO and OAC
Proposed Outcome: An amplicon panel for detection of BO and OAC, and a computational pipeline to convert tissue mutation and methylation data into an optimal panel for any cancer type of interest.
Performed by: Schuster-Böckler lab, Kriaucionis Lab
Objective 3: Evaluate the consistency of the epigenetic features from Objective 2 across BO and OAC tissue samples, and quantify the fraction of BO and OAC tissue DNA in cfDNA.
Proposed outcome: Estimates of the variance of epigenetic marks in BO and OAC, and of the tumour-DNA content in cfDNA. This is an important piece of information not just for determining the necessary sequencing depth, but also to understand the level of cell death in BO in contrast to OAC, and to understand the epigenetic heterogeneity of BO and OAC.
Performed by: Song Lab, Schuster-Böckler lab, Kriaucionis Lab
Objective 4: Estimate the power of targeted TAPS to separate patients from healthy controls.
Proposed outcome: An estimate for the sensitivity and specificity of targeted TAPS as a diagnostic tool in BO and OAC.
Performed by: Schuster-Böckler lab, Song Lab, Kriaucionis Lab

Translational potential
Surgery remains one of the most effective treatments for many solid tumour types, but only if the cancer is detected early enough. Non-invasive diagnostic tests could therefore significantly improve treatment. Oesophageal cancer, in particular, is a devastating disease with an increasing incidence in the UK[4]. Late diagnosis is a key reason why mortality in this cancer type remains high[5]. Genetic and epigenetic changes in the early stages of the disease could be used to stratify individuals into high and low-risk for risk-dependent follow up, and to allow for early intervention.

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9. Identifying novel regulators of pancreatic cancer stem cells via proteomic and single cell transcriptomic methods – Prof. Pauklin[1,2,3]

Primary Supervisor: Siim Pauklin

Additional Supervisors: Udo Oppermann, Zahir Soonawalla.

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: A molecular biology, computational biology, biochemistry, biology or similar background.

Project Summary

Abstract of the project

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies in human due to its highly metastatic characteristics and the poor responsiveness to currently used cancer therapeutics. The dedifferentiation process of cellular identity during tumorigenesis leads to the acquisition of a stem cell-like state of a subpopulation of cells. These are known as cancer stem cells (CSCs), and they are exceptionally important because their developmental plasticity allows them to metastasize and give rise to the whole tumour in the organism. What molecular mechanisms control pancreatic cancer stem cells are currently poorly understood.

Transforming growth factor ß (TGFß) signalling pathway has a central role in tumorigenesis, including pancreatic ductal adenocarcinoma formation. The TGFß-Smad2/3 pathway can stimulate or inhibit cell growth, regulate tissue formation, inflammation, tissue repair, and the stem cell-like characteristics of cancer stem cells (1-4). Our proposed DPhil project will utilize state-of-the-art cell culture methods, single-cell RNA-seq, proteomic analyses, functional and mechanistic studies. Firstly, we aim to identify the transcriptomes of primary PDACs by single cell RNA-sequencing analyses. Secondly, we will uncover novel transcriptional regulators in CSCs of PDACs. Thirdly, we aim to provide characterization of selected new transcriptional cofactors and their cooperation with Smad2/3 on CSCs by functional and mechanistic studies.

The discoveries from this research will identify the transcriptomic heterogenicity of primary PDACs including the CSC subpopulation, and characterise novel transcription factors controlling the cellular characteristics of CSCs. The machineries that govern CSC self-renewal via the TGFß/Nodal-Smad2/3 pathway can be used as novel therapeutic targets for specifically eliminating the metastatic cancer-forming cells in PDAC patients and expand the opportunities for earlier pancreatic cancer detection.

Research objectives and proposed outcomes

(i) the academic value of the research. The objectives of the proposed project are as follows. 1. Identify the transcriptomes of primary PDACs by single cell RNA-sequencing analyses. 2. Uncover novel regulators of CSCs by proteomic analyses. 3. Characterize the function of Smad2/3 transcriptional/epigenetic regulatory cofactors. The research outcomes will be:

- Single cell RNA-sequencing data of tumours from primary PDAC patients.
- Smad2/3 binding candidates co-immunoprecipitating with Smad2/3 in CSCs. Candidate factors will be identified by mass-spectrometry.
- CRISPRi PDAC lines with inducible knockdown (iKD) of selected Smad2/3 cofactors. Our preliminary data has already discovered interesting epigenetic regulators and transcription factors.
- Cell cycle analysis with colony formation data for candidate loss-of-function and gain-of-function experiments.
- RNA-sequencing / ATAC-sequencing / ChIP-sequencing data indicating the phenotype/transcriptomic effects of candidate iKDs.

(ii) Collaborators from different disciplines. The award will help bringing together stem cell and cancer researchers (Siim Pauklin), computational biologists (Udo Oppermann), proteomic specialists (TDI mass-

spectrometry facility), and clinical oncologists at NHS Oxford University Hospitals with access to primary pancreatic tumours (Zahir Soonawalla).

Translational potential of the project

Relevance of the project to cancer. Our research will identify cellular heterogeneity of PDACs and key mechanisms responsible for regulating the pancreatic cancer stem cell identity in PDACs.

- PDAC cellular heterogeneity and CSCs. Elucidating the cellular heterogeneity of PDAC subpopulations will help to identity the extent to which stem cell-like cells known as cancer stem cells might mediate this cancer. Knowing these gene expression profiles will help to devise earlier detection methods for PDAC, and identify gene circuitries that can be used as targets for disrupting the cancer stem cell supporting signals and thereby help eliminate these cells in patients.
- TGFβ/Activin-Smad2/3 cofactors. Understanding the precise molecular mechanisms by which TGFβ/Activin-Smad2/3-cofactor circuitries that govern gene expression in CSCs will allow developing novel therapeutics that will specifically disrupt the functionality of transcriptional complex in CSCs. This will result in destabilizing the stemness network in CSCs, which in turn will lead to the loss of their self-renewal capacity, reduce stem cell-like plasticity and metastatic characteristics.

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10. HPF1 as a biomarker for PARP inhibitor sensitivity – Prof. Ahel[1,2,3]

Primary Supervisor: Ivan Ahel

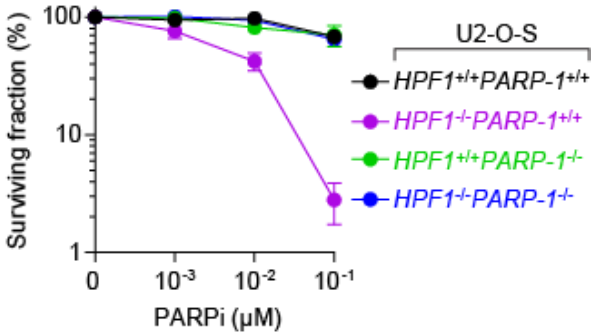
Additional Supervisors: Eric O'Neill

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: Either a biomedical/biological, computer science, mathematical or engineering background.

Abstract

To protect the genome from damage organisms have evolved a cellular defence mechanisms 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]. We have recently identified a novel interactor and regulator of PARP1 ADP-ribosylation activity upon DNA damage, which we have named HPF1[2]. HPF1 forms a robust protein complex with PARP1 in cells and is recruited to DNA lesions in a PARP1-dependent manner. 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. We further demonstrated that HPF1 is a critical specificity factor that allows modification of target proteins by PARP1 on serine residues (Ser-ADPr)[3,4]. Crucially, our work also identified ARH3 as a hydrolase which specifically removes Ser-ADPr[5] and further showed that Ser-ADPr is the major form of ADP-ribosylation following DNA damage[6]. Taken together, our insights surrounding Ser-ADPr open a large, exciting, and novel area of research into the fundamental understanding of the pathways regulated by this modification. Notably, human cancer cells lacking HPF1 exhibit striking sensitivity to PARP inhibitor (PARPi) treatment, see graph right[2]. In addition, unbiased high-throughput screens identified HPF1-deficient cells as sensitive to PARPi treatment[7]. Currently, PARP inhibitors are used to treat breast and ovarian cancer, with recent data suggesting benefits for treating many other cancer types. We therefore propose to test the hypothesis that HPF1 expression might be a useful diagnostic tool with which to stratify cancer patients into sub-groups that will be sensitive/resistant to PARPi treatment. The mechanism of sensitivity of HPF1 cells to PARPi is unknown. Elucidating this mechanism will be another goal of this proposed work.



Research objectives and proposed outcomes

Objective 1. Characterise the effect of HPF1 under- and overexpression in a series of model and primary cancer cell lines on PARP inhibitor sensitivity/resistance. We will collect and test a variety of cancer cell lines, profiling them for HPF1 protein expression levels and then treating with several different PARPi of varying PARP-trapping capabilities (olaparib, talazoparib, veliparib). The commercially available cell lines will be obtained from cell bank repositories and through collaborations. In particular, we will monitor the pancreatic cell lines in a collaboration with Dr. Eric O'Neill (co-investigator in the Primus clinical trial of the PRECISION-Panc consortium).

Objective 2. Elucidating the mechanistic basis for the sensitivity of HPF1 deficient cells to PARPi (modulation of the PARP-trapping, regulation of DNA repair pathway choice, regulation of the chromatin structure/epigenetic marks) and to understand why the loss of PARP1 leads to a resistance (Figure 1). For these studies we will use largely cell biology/biochemical and genomics approaches.

Translational potential of the project

Our data suggest that HPF1 protein expression levels in cancer patients might be a marker that confers sensitivity/resistance of the tumour to PARP inhibitor, providing a rationale for using PARPi for certain patients. Furthermore, as HPF1 loss sensitises cancer cells to PARP inhibitor mediated death, it stands to reason that HPF1 could be a potential small molecule drug target.

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7, Bajrami et al, 2014 Cancer Res 74, 287.

11. Mechanisms of DNA repair and genome stability – Dr. Gibbs-Seymour[1,2,3]

Primary Supervisor: Ian Gibbs-Seymour

Additional Supervisors: None stated

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: A biomedical/biological background.

Project Summary

Abstract
Organisms that encode their genetic information in DNA must ensure that this information does not become corrupted, or damaged, in order to survive and propagate. To safeguard our DNA each one of our cells contains the information to produce molecular machines called proteins. A large number of these proteins are involved in maintaining the integrity of the genome via a process termed DNA repair. DNA repair proteins can sense when and how the DNA is damaged and switch on mechanisms to repair the damage. Importantly, defects within the DNA repair process leads to a range of pathologies and various cancers, so understanding DNA repair is of fundamental importance. We have recently discovered a number of novel DNA repair proteins, the functions of which we are only just beginning to understand. Here, we will build on these exciting discoveries and begin to define the function of these novel DNA repair proteins at a much deeper mechanistic level to help us understand their role in cancer. To accomplish this, we will use a multidisciplinary approach using biochemistry, cell biology and genetics. Through the training of junior researchers in these approaches, I fully expect that the implementation of these approaches will have a transformative impact both their future career and also on our ability to make cutting-edge discoveries. Recently, inhibiting DNA repair processes has become an attractive anti-cancer strategy. The project described here will also allow us to begin the first steps towards finding small molecule inhibitors of these recently identified DNA repair proteins. Collectively, the results will go beyond the current state-of-the-art in the field and provide novel mechanistic insights into DNA repair, providing a deeper understanding of these processes that will ultimately inform therapeutic anti-cancer strategies.

Research objectives and proposed outcomes
The human genome dedicates approximately 500 proteins to DNA repair mechanisms, illustrating the depth of complexity of this cellular process. Cells use signals, called post-translational modifications (PTMs), to instruct these DNA repair proteins where to go and when, in a highly co-ordinated manner. One of these PTMs is called ubiquitination, which involves the addition of ubiquitin, itself a small protein, to target substrate proteins. Ubiquitin may act as an SOS signal for repair proteins to fix the DNA damage and can also be attached to DNA repair proteins to regulate their activity. Ubiquitin signalling becomes more complex due to the ability of one ubiquitin protein molecule to be attached to another ubiquitin molecule, allowing ubiquitin to form chains. However, these ubiquitin signals also need to be turned-off after the DNA repair process is complete. For ubiquitin, this is achieved by a class of enzymes called deubiquitinating enzymes or DUBs. There are approximately 100 DUBs in the human genome that can be divided into 6 different classes. We recently discovered a novel 7th DUB class, which is comprised of just one protein, which we named ZUP1 (Kwasna et al., 2018, Mol Cell). ZUP1 can cleave a certain type of ubiquitin chain called 'K63-linked' ubiquitin chains. K63-linked ubiquitin chains regulate DNA repair processes and indeed I found that ZUP1 is involved in mammalian DNA repair. Now, in order to gain a deeper understanding of ZUP1 function, there are fundamental questions that need to be to addressed to help us understand its role in normal and cancer cells. Therefore, the prospective DPhil student will be trained in either biochemical (B) or cell biological/genetic (C/G) approaches to help us answer these questions.

Research objectives

1. Determine the substrates of ZUP1 using convergent proteomic approaches (C/G).
2. Define genetic interactors of ZUP1 using CRISPR-Cas9 genome-wide approaches (C/G).
3. Use mechanistic cell biological assays to define its cellular function (C/G) in normal and cancer cells.
4. Define ZUP1 function by dissecting how distinct protein domains and post-translational modifications contribute to its enzymatic activity and specificity (B).
5. Understand how ZUP1 deubiquitylates its substrate(s) by *in vitro* reconstitution of the reaction and determination of the structure of the complex (B).
6. Optimise a scalable assay for small molecule inhibitor screening (B).

Proposed outcomes and academic impact of the project

By addressing the research objectives above, we will generate novel mechanistic insights into a recently discovered enzyme family that functions in DNA repair pathways, with relevance to cancer. The academic impact of the project will be achieved by publication of our findings in high-impact journals, through the potential discovery of small molecule inhibitors for cancer therapies, and through the training and mentoring of junior scientists that join the laboratory.

Translational potential of the project

Targeting the ubiquitin system in various cancer settings is becoming a major avenue of drug discovery and oncology research. Thus, inhibiting ZUP1 using small molecules might be therapeutically attractive in the right genetic setting (which we will define using the CRISPR-Cas9 screening). We will begin to optimise a scalable assay using recombinant ZUP1 and a fluorogenic substrate, which will then be utilised for high-throughput screening.

12. Functional translation of MPNST driver gene variants using single cell sequencing and CRISPRi models – Prof. Hassan[1,2,3]

Primary Supervisor: Bass Hassan

Additional Supervisors: Adam Mead, Joey Riepsaame, Robert Kelsh, Damon Reed, Pancras Hogendoorn, Omer Dushek, Charitable Foundation – the Grenfell-Shaw family

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: A biomedical/biological background.

Project Summary

Hypothesis

The identification of the functional co-driver targets at the single cell level, both during the evolution and resistance to therapy of malignant peripheral nerve sheath tumours, will inform novel therapeutic strategies (**Fig 1**).

Abstract

Cancers are derived from rare undifferentiated cell types that display multi-potency (cancer stem cells, CSC) that when combined with positive selection of driver mutations, result in multi-level intra-tumour heterogeneity [1]. Strategies to improve treatment outcomes are therefore dependent on targeting the cell of origin and the 'personalised' combinations of driver variants that evolve during treatment selection pressure at the cellular level. Migration of neural crest cells (NCC) into all organs during development gives rise to Schwann cell progenitors (SCP, peripheral nerves and ganglia), melanocytes and mesenchymal cells (**Fig. 2a**). NCCs are the 'cells of origin' for multiple cancer types, e.g. melanoma, neuroblastoma, phaeochromocytoma, medullary carcinoma of the thyroid, peripheral neuroectodermal tumour (Ewing sarcoma), melanocytic neuroectodermal tumours of infancy (MNT) and malignant peripheral nerve sheath tumours (MPNST) [2] (**Fig. 2b**). Here, we aim to perform unprecedented functional evaluation of MPNST (a sarcoma of unmet need) following single cell genomic analysis of human tumours using cutting edge technologies that, for the first time, will allow deep mining for driver variants and their evolution. We will then model drivers using multi-targeted CRISPRi technology, and rapidly translate these findings into personalised approach, from novel tumour biomarkers to combined agent and sequential therapeutic strategies.

Purpose of the Research

NCC derived cancers explain the overlapping similarities in diagnostic biomarkers for melanomas and MPNSTs (S100 and KROX2) and, as we have shown, expression of tyrosinase in rare MNTs [3,4]. Here, we focus on frequently fatal MPNSTs often associated with Neurofibromatosis type 1 (NF-1, Von Recklinghausen's disease). NF-1 is an autosomal dominant disorder with a prevalence of ~ 1 per 3000. Heterozygous loss of function variants in NF-1 (neurofibromin), a RasGAP that negatively regulates Ras, results in atypical neurofibroma (ANF) and complex plexiform neurofibroma (PN), with associated cellular infiltration (fibroblasts, mast cells, dendritic cells). The functional mechanisms of transformation of ANF/PN to MPNST, including how non-NF-1 and sporadic

MPNST's (50%) develop, remain unknown [5]. There are numerous advantages for taking a single cell sequencing

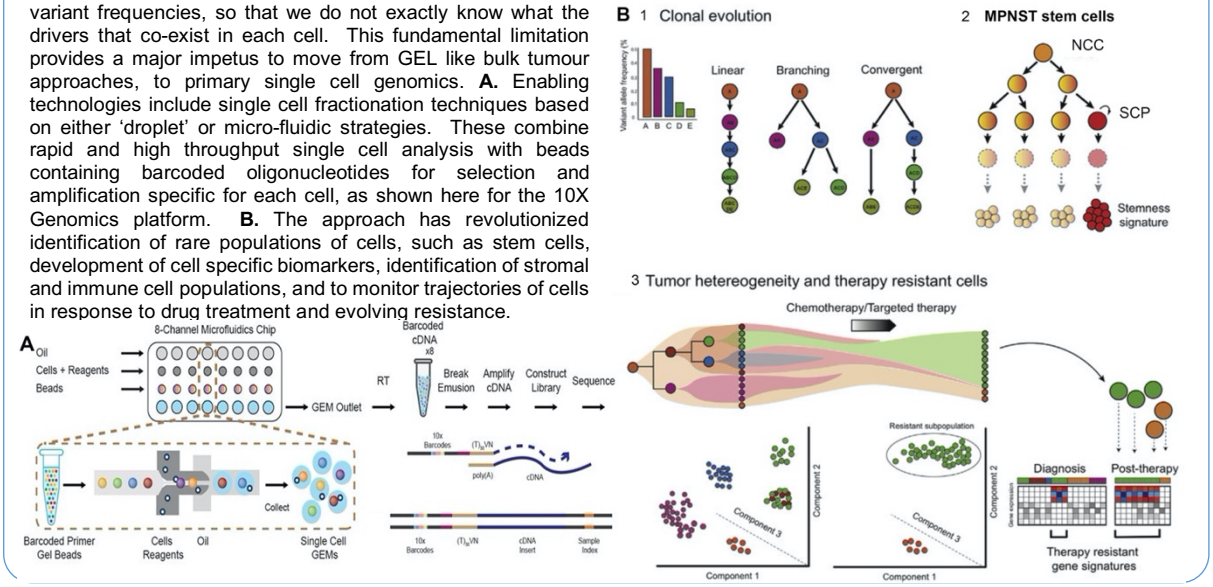


Figure 1. Primer of single cell sequencing. Whole genome approaches of bulk tumours lead to inferred estimation of allelic variant frequencies, so that we do not exactly know what the drivers that co-exist in each cell. This fundamental limitation provides a major impetus to move from GEL like bulk tumour approaches, to primary single cell genomics. **A.** Enabling technologies include single cell fractionation techniques based on either 'droplet' or micro-fluidic strategies. These combine rapid and high throughput single cell analysis with beads containing barcoded oligonucleotides for selection and amplification specific for each cell, as shown here for the 10X Genomics platform. **B.** The approach has revolutionized identification of rare populations of cells, such as stem cells, development of cell specific biomarkers, identification of stromal and immune cell populations, and to monitor trajectories of cells in response to drug treatment and evolving resistance.

approach (See **Figure 1** for a primer of single cell sequencing), particularly as there has been a step change in the platform technologies available. The cutting-edge status of the technology and its applications are now being rapidly applied to personalised therapeutic selection directed at driver variants and immune cell targets (**Fig. 1**).

Here, this CRUK DPhil studentship that will work jointly with a post-doctoral scientist in order to develop a cohesive clinical science strategy in the genomics of MPNST. This project is built on existing strengths in single cell genomics in Oxford (MRC Weatherall Institute of Molecular Medicine Single Cell Genomics facility- Prof Adam Mead). We will exploit stem cell and genome engineering technology (lead by Dr. Joey Riepsaame) at the Sir William Dunn School, with translational pathology and clinical trials embedded in European networks. In order to develop this translational approach for MPNST, matched funding from the *Grenfell-Shaw* charitable trust that will support a dedicated post-doctoral research scientist throughout the studentship, maximizing the potential for success and seeding future critical mass with the existing single cell sequencing consortia in Oxford and disseminating experience of the approach to the cancer community.

Background and Rationale

Identification of NF1 heterozygosity combined with variants in RAS-ERK, Tp53 and WNT pathways in MPNST have not fully explained mechanisms of initiation, progression and novel therapeutic strategies [6]. ANF/ PN are derived from adult late stage immature Schwann cell precursors (iSCP), that derive from embryonic Schwann cell precursor cells (SCP) and NCC [7]. Primary mutations in NF-1 are followed by 2nd hits, but may not be sole drivers of MPNST progression. Importantly, recent somatic NGS identify loss of function of the polycomb repressor complex-2 (PRC2) components EED and SUZ12, which is unlike myeloid malignancies that frequently also mutate EZH1/2 [8,9]. PRC2 normally epigenetically regulates silencing through histone H3 lysine methylation (H3K27me2/3). As a result of loss of methylation and repression, transcriptional activation of interferon and growth pathways appear to then establish vulnerabilities to chromatin targeted agents (e.g. HDACs) [10]. Moreover, NGS has identified potential common variants associated with ANF/PN transitions (CDKN2A loss, PRC2 mutations) [11,12]. Sleeping beauty mouse models validated additional driver pathways such as EGFR, p16yclinD-CDK4-RB and Tp53-MDM2 [13]. Neurofibromin has other functions, as a regulator of RHO/ROCK signaling through cytoskeletal interactions, suggesting that motility and angiogenesis are co-regulated [14]. The challenge is to identify the cells of origin, the clonal landscape of MPNST driver mutations and cell type gene expression and rational therapeutics.

Objectives

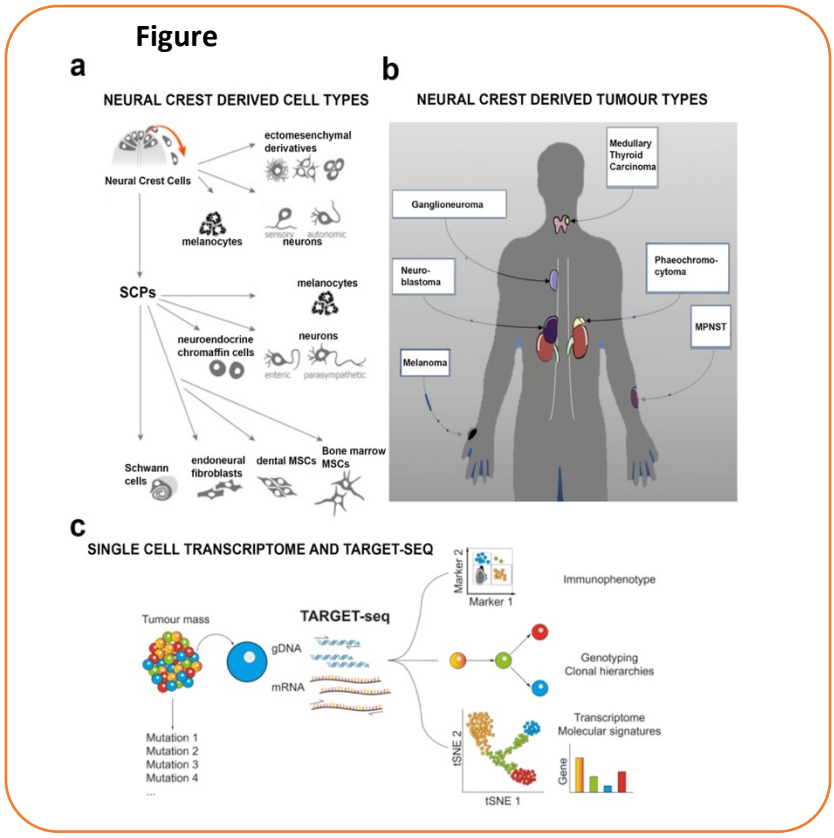
1. Combine unbiased single cell transcriptome, variant heterogeneity and epigenome in human primary MPNSTs and xenografts (Year 1-2).

2. Analysis of evolution of MPNST driver variants, including infiltrating cell types (Year 2).

3. Evaluate the functional basis of driver variants alone and in combination, including with targeted agents, using CRISPRi in model systems (year 3).

Plan of Investigation and Methods to be Employed

1. Combine unbiased single cell transcriptome, variant heterogeneity (scTARGETSeq) and epigenome in human primary MPNSTs (Oxford Sarcoma Service) and xenografts (PDX) (Year 1-2). Single cell RNA sequencing (scRNASeq) of thousands of cells, for example using barcoded Drop-Seq [15] and poly-A capture methods [16] and have paved the way for detailed characterization of unbiased transcriptome heterogeneity in tumours. For the first time, we will also apply scTARGET-Seq methodology to sarcoma, and combine deep coverage of libraries of genomic and somatic variants, so minimizing allelic dropouts [17,18] (**Fig. c**). Conventional scRNA-seq protocols do not allow reliable mutational analysis, whereas scTARGET-seq combines high-sensitivity genomic DNA and cDNA genotyping with scRNA-seq. scTARGET-seq also resolves the distinct transcriptional signatures of tumor genetic sub-clones and non-mutant cells from patients that may show aberrant immune-associated gene expression. We will collaborate closely with the Mead lab at the WIMM to perform the methodology and analysis, initially using MPNST derived cell lines in order to optimize the method, and with the Reed lab in Tampa, in order to fully evaluate existing WES whole-tumour MPNST variants [11]. We will first optimize single cell disaggregation and microfluidic methods using the gentleMACS™ and 10xChromium™ platforms, [19]. We will optimize gentleMACS™ sample preparation that include red cell and 2xdead cell removal protocols [20]. We may also use NCS markers (p75^{NTR}-CD271+i) to enrich for progenitor populations, before collecting fresh tumor material from theatre (5-8 x large volume high-grade NF-1 associated and non–associated MPNST resected per year, Oxford Sarcoma service). Single cell dimensionality reduction will be performed using standard 10xChromium™ tSNE package, as well as informatics tools within the existing single cell consortia bioinformatics collaborators.



2. Analysis of evolution of MPNST driver variants, including infiltrating cell types (Year 2-3). We will next apply scTARGET-Seq informatics methodology in experiments through collaboration with the post-doc and Mead lab informatics core. Using unbiased transcriptome analysis in ~5-10,000 cells per tumour, we will identify cell types using principle component analysis methods, including potentially CSC based on iSCP and NCC expression signatures. We will then combine these data with target sequencing for driver variant data, to resolve the evolutionary order and heterogeneity within MPNSTs at the single cell level [18]. By using 5' processing on the 10xGenomics platform that better captures V(D)J recombination events, we will sequence TCR□/□ chains to determine clonal T-cell populations and extent of immune diversity. With the Dushek lab (SWDS), we will descriptively and mechanistically model antigen specific T-cells using these data. Using such combined analysis, we will identify variant associated biomarkers that can be applied in patient selection and therapeutic response evaluation (collaboration with Hogendoorn, Sarcoma Pathology, LUMC), as well as co-regulated pathways, such as innate immunity gene sets (GSEA) and cell cycle expression.

3. Evaluate the functional basis of driver variants alone and in combination, including with targeted agents, using CRISPRi in model systems (year 3). MEK inhibition has had modest activity in clinical trials of MPNST [21], despite preclinical evidence [22], and current trials are focused on agent combinations, such as mTOR and HSP90 inhibition (Sarc023: Ganetesib and sirolimus in MPST NCT02008877). If patients develop metastatic disease, and undergo further treatment such as chemotherapy, we will aim to obtain further tumour tissue under informed consent for serial biopsies, in order to directly sample tumours for scTARGETSeq specific for the variants identified in their primary sample. By deeply mining data from a small number of patients, we will investigate variant selection under such selection pressure. We will also identify candidate drivers in primary tumours and model their profiles and function in MPNST cell lines (e.g. S462TY, ST8814, ST883, 908, NF02.2, T265, SNF96.2). By using CRISPRi with inducible Cas9 fusion genes, we will complement existing variants with induction of gain or loss of function variants for specific combinations (Genome Engineering, SWDS). By barcoding cell lines with specific additional induced variants, we will test co-variant selection *in vitro* in combined cultures, and *in vivo* in xenograft models [23]. To further characterize function in the context of combined drivers, we may also utilize iPS cells with Noggin/SB431542 selection with CRISPR targeting to isolate Schwann cell precursors. As zebrafish models recapitulate NCS-melanoma-MPNST biology [24], we will also collaborate with Robert Kelsh (Bath) with respect to the isolation and differentiation of neural crest stem cell towards melanoma and Schwann cells (SOX10+) precursors [25], especially with flow cytometry [26], prior to CRIPR/Casp variant modification. If driver targets also have selective drugs available, we will test the single agents and combinations of agents for synergistic activity using screens in culture and in xenograft models.

Translational potential: This project is a step towards a deeper understanding of MPNST biology. These steps are essential as they will underpin biomarker cell type validation and variant selection in individual patients with MPNST. The approach would contribute a step-change to enable the integration of single cell sequencing technology into early phase clinical trials, and so the rationale design of sequential single agent and combination targeting clinical trials that are truly personalised in MPNST patients. The student will be based at the Oxford Molecular Pathology Institute, Sir William Dunn School (Hassan & Riepsaame) and the Oxford Single Cell Sequencing Consortia (Mead-WIMM) and will have opportunity participate in the MPNST translational clinical service (Prof Hassan, Medical Oncology, Oxford Cancer and Haematology Centre).

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13. Targeting histone H3K36me3 deficient cancers - Prof. Humphrey[1,2,3]

Primary Supervisor: Timothy Humphrey

Additional Supervisors: Bart Cornelissen

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: Either biological, biomedical, biochemical, chemistry or pharmacology background.

Project Summary

Abstract
SETD2-dependent trimethylation of histone H3 lysine 36 (H3K36me3) is frequently lost or depleted in particular cancer types, including kidney (where it is lost in up to 60% of metastatic cases), identifying this histone mark as an important therapeutic target. We identified a novel genetic way (synthetic lethality) in which H3K36me3-deficient cancers can be targeted using the WEE1 inhibitor AZD1775 (MK-1775) (Pfister et al Cancer Cell, 2015), which has now entered clinical trials. With the aim of overcoming possible resistance to AZD1775, we have screened for other synthetic lethal agents that target loss of this histone mark, and have identified a novel agent that specifically targets H3K36me3-deficient cancer cells, at nanomolar concentrations, through an as yet unknown mechanism. Here, we propose to identify the drug target(s) using azide/photo-affinity linker approaches (**Aim 1**); validate the targets (**Aim 2**); use these targets to develop structure-activity relationships to generate functionally analogous molecules, including fluoride-tagged variants, allowing the development of an ^{18}F-radiolabelled variants for PK/PK studies using PET imaging (**Aim 3**); and analyze these novel ligands to determine whether they are functionally equivalent (**Aim 4**). This research will provide novel insights into the function of H3K36me3, identify novel targeting mechanisms, and facilitate the rational development of a novel class of drugs to target H3K36me3-deficient cancers. Further details will be made available at interview.

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14. Developing a highly multiplexed single B cell resolution antibody specificity assay (HiMAbSpec) for investigating the B cell immune responses to tumour neo-antigen – Dr. Bashford-Rogers[1,2,3]

Primary Supervisor: Rachael Bashford-Rogers

Additional Supervisors: Sarosh Irani, David Church, David Mole, Adam Baily

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: Either a biomedical/biological, computer science, mathematical or engineering background.

Project Summary

Abstract
B cell infiltration has prognostic significance in solid tumours, and ongoing studies are investigating their phenotypes through single cell transcriptomics and spatial imaging. Characterising the B cell response to tumour cells, particularly their antigenic specificities, will be key to developing more immunologically appropriate cancer therapies. However, currently, the B cell antibody (Ab) specificity cannot be coupled with the Ab sequence, phenotype or transcriptome in a high-throughput manner. Here we propose a DPhil studentship project to develop novel technologies to be able to bridge the gap between B cell populations and antibody reactivity, thus giving a unique perspective on the development of anti-self, anti-tumour and anti-non-self Ab responses. Through understanding the role of Abs and the nature of antigen specificity of B cells both within the tumour and within the periphery, this may potentially positively impact the lives of patients through highlighting potential novel B cell-associated targets for therapy. Furthermore, this method is not just broadly applicable to cancer, but could have wider applications in immunology and biotechnology.

Background, Significance and Innovation
Given the clinical efficacy of immune checkpoint inhibitors in advanced melanoma and several other solid tumours[1,2], there is a growing appreciation of the role of B and T cells in modulating immune responses to many tumours. The generation neo-antigen by tumour somatic variation has been shown to confer tumour immunogenicity and induce anti-tumour responses[3]. Indeed, the diversity, function and interplay of different B and T cell responses in solid tumours are not understood, particularly in concert with tumour genetics. We have previously shown that an integrated genomic and immune multi-platform profiling approach in therapy-resistant breast cancer has been insightful by demonstrating metastases propagate and evolve as communities of clones (De Mattos-Arruda et al., Cell Reports 2019). Through analyses of the repertoire of T cell receptors in these multiple individual metastases, we have revealed significant levels of immuno-surveillance between metastatic sites, and that the adaptive immune responses appear to co-evolve with the metastatic genomes. There is also accumulating evidence for an important role of B cells in modulating immune responses to both solid tumours and haematological malignancies and the association with prognosis. Breg subsets can suppress T-cell and natural killer cell responses. This is supported by the finding that increased density of infiltrating B-cells and activated T-cells in human melanoma is associated with favourable prognosis[2] and that B-cell depletion significantly decreases tumour growth[1]. Previous studies have shown that patterns and levels of tumour infiltrating B-cells IgG H chain somatic hypermutation suggested affinity maturation in intra-tumoural germinal centres[4]. However, the potential contributions of B cells to the immune response to tumour development are less well investigated, particularly in relation to B cell antigen-specificity. Here, we will develop a novel high-throughput method to probe the antigen specificities of B cells, which will be coupled with information about the B cell clonal phenotype and transcriptome at single cell resolution. We will use this to investigate the development and role of tumour-infiltrating B cells across a range of tumours with varying degrees of immunogenicity. This study provides a unique platform to understand the probe between tumour neo-antigen,

B cell immune-surveillance and specificity, and B cell phenotype, with the overall aim of highlighting new therapeutic options.

Vision and aims

We aim to develop a novel method to investigate the B cell immune response of circulating and tumour-infiltrating B cells across a range of tumours with different levels of immunogenicity and neo-antigen expression. Not only will this provide information about the range of specificities per B cell, but also the range of Abs that are specific to each tumour cell at a single-tumour cell resolution. We will use the Ab sequence, which is a molecular barcode for each B cell clone, to link B cell clonal phenotype and transcriptome to the Ab specify. This may provide a powerful platform to answer key questions in the field of tumour immunology including:

- Do tumour-associated B cells produce Abs against tumour cells, and how does tumour cell neo-antigen variation and expression level dictate Ab response? How is this related to serum reactivity?
- What is the antigen specificity of different tumour infiltrating B cell subsets, and what is the relationship between pro-tumour (such as IL10-producing B cells) and anti-tumour B cell subsets? What determines the balance of whether B cells serve a pro- or an anti-tumourigenic function? Are tumour-associated Abs cross-reactive to other self or non-self antigen, poly-reactive, or highly specific to tumour cells?
- What is nature of escape tumour cells (i.e. tumour cell 2 where few/no Abs bind) compared to tumour cells with polyclonal Ab responses (e.g. tumour cell 3) and specific tumour cell responses (e.g. tumour cells 1 and 4)? Do different neo-antigens elicit different B cell responses (differences in isotype/phenotype) and what are the tumour cell determinants of Ab immunogenicity? What is the association with other cell populations? What is the variation over the course of therapy and association to prognosis/ relapse?

Overall, this may help shed light on the B cell response to tumour cells, the specificity and breadth of response, and potentially highlight novel therapeutic targets. We envisage that this novel platform may be extended to the other areas of B cell biology, and could be a general tool that could be of great value to Cancer Centre researchers or industry in determining the high-throughput B cell antigen specificity. This project is made possible by 3 key innovations:

1. Development of single cell secreted Ab labelling through oligo-conjugated secondary Ab coupled with established B cell *in vitro* differentiation/secretion methods, thus preserving native Abs post-translational modifications.
2. Using B cell receptor sequencing to link known Ab specificities with B cell phenotype through clonal relatedness, and hence better understand the interrelationships of the infiltrating and circulating B cells.
3. Published (and *in house* validated) digestion protocols that faithfully release cells from tissues in order for scRNA-seq in both fresh and frozen samples[5] and bioinformatic methods to minimise batch effects[6].

Objectives

Work-package 1. Optimisation of highly multiplexed single B cell resolution specificity protocol (HiMAbSpec). First methodologies and reagents will be developed and optimised, including as oligo-labelled secondary Abs, generation of cell lines and recruitment of positive/negative control samples. We will first test the reproducibility, specificity and sensitivity of the HiMAbSpec method through dilution assays on cell lines expressing known antibodies and on peripheral blood samples from patients with known clinically-associated auto-Ab specificities.

Work-package 2. Perform coupled HiMAbSpec & Ab sequencing on circulating B cells against tumour cells. Once optimised, HiMAbSpec will be performed on tumour tissue samples. Tumour samples will be recruited based on level of tumour immunogenicity and neoantigen (e.g. DNA mismatch repair proficient vs. deficient vs. *POLE*-mutant carcinomas) for an initial comparison. In conjunction with HiMAbSpec, the remainder of tumour-infiltrating B cells will undergo scRNAseq analysis using 10X Chromium (5' transcriptome & B cell VDJ sequencing), and matched peripheral blood will be cell sorted into key B cell subsets, and isotype-resolved bulk-cell Ab sequencing[16] will be performed. Targeted sequencing of key loci in the tumour cell populations will be performed to couple with the Ab data.

Work-package 3. Integration of Ab specificity, Ab sequence, scRNAseq & tumour genetic variation. Each dataset will be integrated based on shared Ab sequences, which are molecular barcodes for each clone and defines Ab specificity. Clonal sharing and dynamics will be estimated by phylogenetic inference through an extension of methods in [7]. Models of tumour & B cell selection, dynamics and phenotype will be generated. This can be expanded to pre-

malignant lesions, during immunotherapy and/or in metastasis for a more generalisable model of B cell tumour responses.

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15. Tissue and Single-cell Mass Spectrometric Imaging for Brain Cancer Precision Medicine – Dr. Ansorge[1,2,3]

Primary Supervisor: Olaf Ansorge

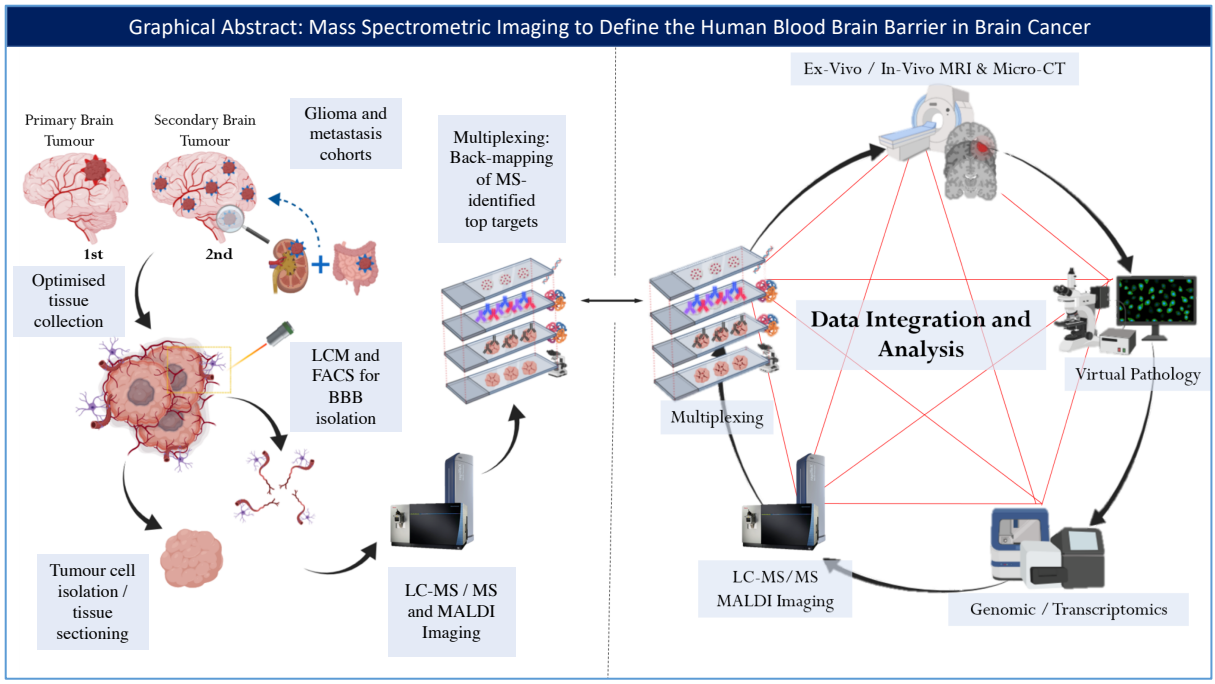
Additional Supervisors: Claire Vallance (Co-PI), Roman Fischer, James McCullagh, Alistair Easton, Sarah Blagden, Josephine Bunch, Gary Kruppa

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: Medicine, biology, physical chemistry, biochemistry, engineering or bioinformatics.

Project Summary

We are offering an exciting multidisciplinary research project at the interface of human brain cancer biology and mass spectrometry imaging (MSI) technology [1, 2]. Specifically, the student will use human brain cancer tissue obtained at surgery in clinical trials to define the proteome and metabolome of the blood-brain and blood-tumour barrier (BBB/BTB) at whole tissue and single-cell resolution. The BBB/BTB controls access of therapeutics to the brain, and its specific barrier function may be related to brain cancer genotype [3]. Here, we will focus on gliomas characterised by mutations in isocitrate dehydrogenase (IDH) and brain metastases from renal cell carcinoma. Both cancers are associated with profound, partially overlapping, metabolic alterations characterised by accumulation of 'oncometabolites' (e.g. D- or L-2-hydroxyglutarate) [4-6]. How the altered brain cancer metabolome relates to the proteome and structural integrity of the human BBB/BTB is unknown. The challenge of this project is to develop workflows suitable for 'deep' tissue proteome and metabolome analysis and to digitally integrate results with complementary data derived from genetics, histology and magnetic resonance (MR) [4] or computed tomography (CT) imaging. Cell-enrichment techniques such as fluorescent activated cell sorting (FACS) or laser capture microdissection will be combined with 2D and 3D tissue section imaging using the latest MSI technologies [2]. This work will contribute to international brain cancer atlas projects and establishment of MSI as a diagnostic tool in cancer tissue analytics.



The student will join a new translational 'neuroproteomics' group at Oxford involving the Nuffield Departments of Clinical Neurosciences and Medicine (Target Discovery Institute) and Department of Chemistry, and benefit from affiliations with the National Physical Laboratory and Bruker Daltonics, a leading MSI company. The appointee will also acquire expertise from the EU-funded IM2PACT project (http://im2pact.org/), an Oxford-led industry-academia partnership established to identify novel drug delivery pathways across the BBB in neurodegenerative and inflammatory disorders (but not brain cancer).

Both medical and basic science applicants are welcome. The emphasis of the project will be adjusted according to the successful candidate's scientific background (clinical translation (three years) vs. analytical technology (four years)). Prospective applicants are strongly encouraged to contact olaf.ansorge@ndcn.ox.ac.uk and claire.vallance@chem.ox.ac.uk, principal supervisors of this studentship.

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16. Investigating IGFs as cancer risk factors – Prof. Macaulay[1,2,3]

Primary Supervisor: Valentine Macaulay

Additional Supervisors: Ruth Travis, Tim Key, Sarah Blagden**,** Andrew Protheroe

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: A biomedical/biological background.

Project Summary

Abstract
We are seeking a motivated clinical research fellow (CRF) or non-clinical graduate student for doctoral research that will complement our investigation of insulin-like growth factors (IGFs) as cancer risk factors (Macaulay), and dietary and lifestyle factors that influence cancer risk (Travis, Key). The primary research aim is to identify mediators of high IGF-1 in order to develop novel approaches to risk reduction. In current work we are studying effects of varying IGF supply on epithelial cell populations, based on our recent findings that serum IGF-1 associates with altered expression of the IGF receptor (IGF-1R) in malignant prostatic epithelium, and IGFs regulate RRM2 and dNTP supply, which others have shown can be mutagenic. IGFs are also known to enhance Treg function and secretion of immunosuppressive cytokines. These data lead us to speculate that a high IGF environment drives a pro-mutagenic tumour profile while suppressing the ability to mount an anti-tumour immune response. The student will investigate this hypothesis, using *in vitro* and *in vivo* models and clinical and public data to investigate the relationship between IGF supply and immune cell function. A clinically-qualified CRF will also have the opportunity to contribute to an academic trial of an intervention that aims to delay progression of early prostate cancer, as a step towards development of risk reduction strategies. Training will be provided in the Macaulay and Travis/Key groups and in Uro-Oncology and Early Phase Trials practice by Profs Protheroe and Blagden. This is an exciting opportunity for academic training in basic, translational and clinical research to understand how effects on the tumour micro-environment (TME) contribute to the association of diet and IGF-1 with cancer risk and progression.

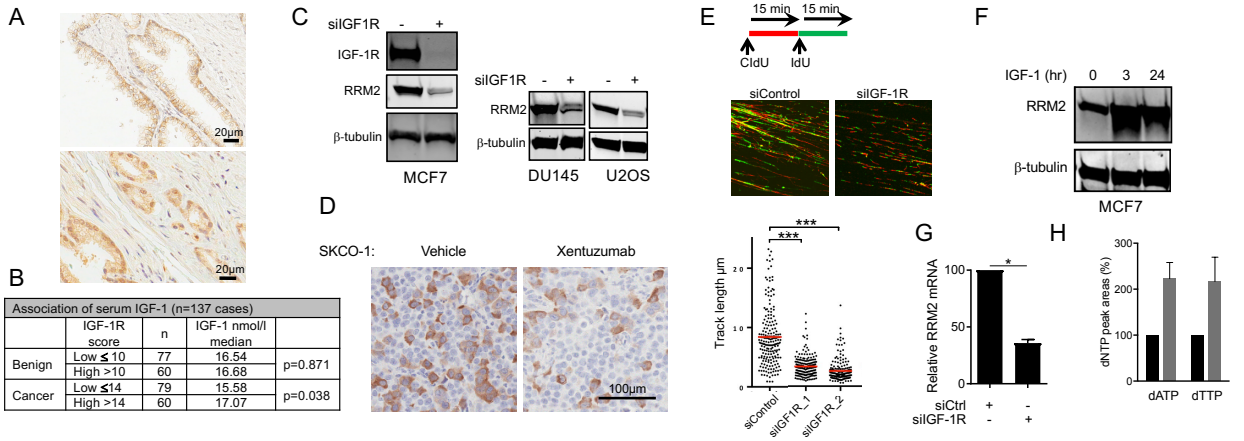
Research objectives and proposed outcomes

Background: Subjects with low serum IGF-1 are strongly protected from cancer (2, 3), while people with high IGF-1 are at increased risk of breast, prostate and colorectal (CRC) cancer (4-6). IGFs are implicated in the association of height with incidence of many solid and haematological cancers, and with aggressive, lethal prostate cancer (1, 7). There is compelling preclinical and clinical evidence that IGF-1 is not just an associated factor, but causative (3, 8). Our long-term aim is to identify mediators of high IGF-1 in order to develop novel approaches to risk reduction. This aim is supported by Cancer Research UK (programme, Travis /Key; Early Detection grant, Macaulay) and Prostate Cancer UK (PCUK, Macaulay).

The current application follows our identification of two novel effects of varying IGF supply in prostate, breast and CRC models and clinical cases (Fig 1). First, in men with early prostate cancer, serum IGF-1 associates with IGF-1R content of malignant epithelium (Fig 1A-B). This is the first clinical tissue-level change linked to serum IGF-1. IGF-induced IGF-1R upregulation is also reported in infiltrating immune cells in murine CRC (9), representing an initial step to understand how IGF-1 affects cancer risk. Secondly, we discovered that IGFs regulate expression of the RRM2 subunit of ribonucleotide reductase, the rate-limiting step for dNTP production. RRM2 is downregulated by IGF-1R depletion or IGF blockade using IGF neutralizing antibody xentuzumab (Fig 1C-D), resulting in delayed progression of the replication fork (Fig 1E). Conversely, IGF-1 upregulates RRM2 at the mRNA and protein levels (Fig 1F-G), leading to dNTP pool imbalance (Fig 1H). In other models, comparable dNTP imbalance is reported to impair proof-reading by the replicative polymerases, leading to increased mutational burden (10). These new findings suggest a novel route through which high IGF-1 induces pro-tumorigenic changes in the tumour epithelium.

This project is motivated by recognition that IGFs also have important roles in regulating the function of components of the TME. IGFs are reported to enhance Treg function and secretion of immunosuppressive cytokines, with clinical

evidence of low immune cell infiltration in high IGF-1R prostate cancer bone metastases (11-13). Furthermore, IGF axis gene downregulation was identified as a critical change in B cells of healthy aged mice (14). We are now collaborating with Professor Len Seymour's group to test effects of varying IGF-1 supply on tumour cells and peripheral blood mononuclear cells, with evidence in tumour cells of dysregulated expression of cytokines and other immune mediators (not shown).



Association of serum IGF-1 (n=137 cases)				
	IGF-1R score	n	IGF-1 nmol/l median	
Benign	Low ≤ 10	77	16.54	p=0.871
	High >10	60	16.68	
Cancer	Low ≤14	79	15.58	p=0.038
	High >14	60	17.07	

Figure 1 IGF-1 associates with IGF-1R content of malignant prostatic epithelium, and regulates RRM2 and dNTP supply. A. IGF-1R immunohistochemistry in upper: benign, lower: cancer areas of radical prostatectomy. **B.** Serum IGF-1 associates with IGF-1R in malignant but not benign prostate. **C, D**. RRM2 is downregulated by IGF-1R depletion in MCF7 breast cancer, DU145 prostate cancer, U2OS osteosarcoma cells and by IGF neutralizing antibody xentuzumab in SKCO1 colorectal cancer xenografts. **F.** DNA fiber assays: significant slowing of replication fork in IGF-1R depleted MCF7 cells. **G-I**. IGF-1 upregulates RRM2 protein (G) and mRNA (H) and increases cellular content of dATP, dTTP (I). Data in C-H from Rieunier et al. IGF targeting perturbs global replication through ribonucleotide reductase dysfunction. Under review.

Aim: to investigate the hypothesis that immune sequelae of high IGF-1 supply contribute to cancer risk.

Experimental plan*:* The CRF will work with a CRUK-funded Post-doctoral scientist and Bioinformatician who are investigating IGF effects on mutation and transcriptional profiles of target epithelia. The CRF will:

1) Test effects of manipulating IGF supply on phenotype, transcriptional profile, function of circulating and tumour-infiltrating immune cells. Depending on progress with current research, areas that may be ripe for investigation include: i) immunoprofiling by flow cytometry, ii) single cell RNA-seq, iii) xCELLigence killing assays, quantifying killing as loss of adherence of breast, prostate or CRC cells when killed by non-adherent effector CD8+ T cells as (15). Assays will use fresh human T cells or cell line TALL-104 cultured ± IGF-1 *in vitro*, and immune cells sorted from blood and murine Myc-CaP prostate cancers established in wild-type vs high IGF-1 mice (16), which are being used in our CRUK project.

2) Contribute to academic trial of IGF blockade, aiming to identify IGF effectors that may be targets for risk reduction. The trial is due to open Q1 2021, and will test 4 weeks' IGF blockade pre-prostatectomy (PI Macaulay, supported by PCUK and Boehringer Ingelheim). Data in Fig 1F and (9) suggest 4 weeks is sufficient to influence RRM2 and profile of tumour-infiltrating immune cells. Trials will generate samples for analysis as 1).

3) Interrogate public data to assess correlations between tumour immune profiles and serum IGF-1, using surrogate SNP markers of serum IGF-1 available from a large-scale genome-wide association study of circulating IGF-I. This approach will allow correlations to be tested in a wide range of solid and haematopoietic malignancies.

Academic value of the research. IGF effects on malignant epithelium are relatively well-characterised. In contrast much less is known about pro-tumorigenic effects of IGFs in the TME. This project has potential to identify proteins and pathways that are novel mediators of high IGF-1 on cancer risk.

Collaborations. This project will cement collaboration between the Macaulay and Key/Travis groups, and will take advice from Len Seymour and from Hashem Koohy, who has expertise in single cell transcriptional and epigenetic profiling of immune cells (14). If clinically qualified, the CRF will be trained in clinical trials procedures by Profs

Blagden and Protheroe. This project represents an exciting opportunity to conduct basic, translational and clinical research at the interface of precision medicine and immunology.

Translational potential. The overarching aim of this research is to identify and block the key mediators of high circulating IGF-1, which may be novel stromal biomarkers of risk, and targets for risk reduction.

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17. Spatial mapping of intercompartmental tissue co-evolution in colorectal cancer – Prof. Leedham[1,2,3]

Primary Supervisor: Simon Leedham

Additional Supervisors: Tim Maughan, Jens Rittscher

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: Either computer science background or willingness to learn computer science.

Project Summary

Abstract Colorectal cancer is a complex ecosystem, dictated by the inter-dependence of three distinct tissue compartments – the epithelium, the stroma and the immune system. These three compartments co-evolve; from maintenance of intestinal homeostasis, through the initiation of precursor lesions and the invasion and dissemination of cancer cells. Recent transcriptome-based molecular phenotyping of CRC has shown that response to therapy and prognosis is also determined by this tri-compartmental interaction (1, 2), yet the majority of first line chemotherapeutics only target the proliferating tumour epithelium. Understanding the intercompartmental cell-to-cell 'rules of engagement' and therapeutically exploiting cell signaling between the tumour microenvironment and the malignant epithelium could represent an important new treatment paradigm in CRC. As we learn more about how the genetically altered neoplastic epithelium, is shaped and constrained by the tumour context, there is a growing need to quantitively assess cancer phenotype 'on-slide'. We have established a multi-disciplinary consortium to integrate large-scale cancer genomic molecular phenotype with tissue morphology. We will use our biological understanding of intercompartmental signaling together with machine learning and multiplex multi-colour imaging analysis to correlate key signaling pathway disruption with histologically detectable change. If successful this will enable on-slide stratification of CRC patients from histological images, and facilitate the spatial analysis of tri-compartmental interaction in CRC and other solid tumours. We are looking for a clinical fellow to work within this team, developing timely image analysis, machine learning and data interpretation skills in a project with direct translational potential.

Background The intestinal mucosa is a complex ecosystem, dictated by the inter-dependence of three distinct but intertwined tissue compartments – the epithelium, the stroma and the immune system. These three compartments co-evolve; from embryonic tissue development, through maintenance of intestinal homeostasis, tissue regeneration and the initiation of pathology. This interaction is regulated by inter-compartmental crosstalk through secreted cell-signaling networks. Disruption of this crosstalk, instigated and punctuated by the accumulation of epithelial genetic mutation, underpins pre-malignant precursor initiation and the transformation to colorectal cancer (Figure 1).

Work from Oxford has shown that single cell molecular interrogation can quantitively assess dynamic and complex stromal (3) , epithelial (4)and immune cell populations in intestinal homeostasis and inflammation. However, as we learn more about how the epithelium, is shaped and constrained by stromal and immune cells, there is a growing need to understand how these cells spatially relate to each other 'on-slide'. By taking an iterative molecular and morphological approach using state-of-art digital pathology, multiplex imaging and machine learning techniques, we will assess lesion phenotype as an output measure of dynamic intercompartmental co-evolution. If we can map cell population ecology and comprehend the signalling pathways that control intercompartmental cell interactions, we have the potential to identify new drug targets for therapeutic manipulation.

Research objectives. We hypothesise that inter-compartmental crosstalk regulates cellular interaction in homeostasis and is co-opted and corrupted in cancer. We believe that mapping cell interaction as tissue progresses from normal to pathology can help define cell-to-cell 'rules of engagement'. This will allow us to make sense of the apparent cellular heterogeneity of tumours, and identify potential novel intercompartmental signalling therapeutic targets.

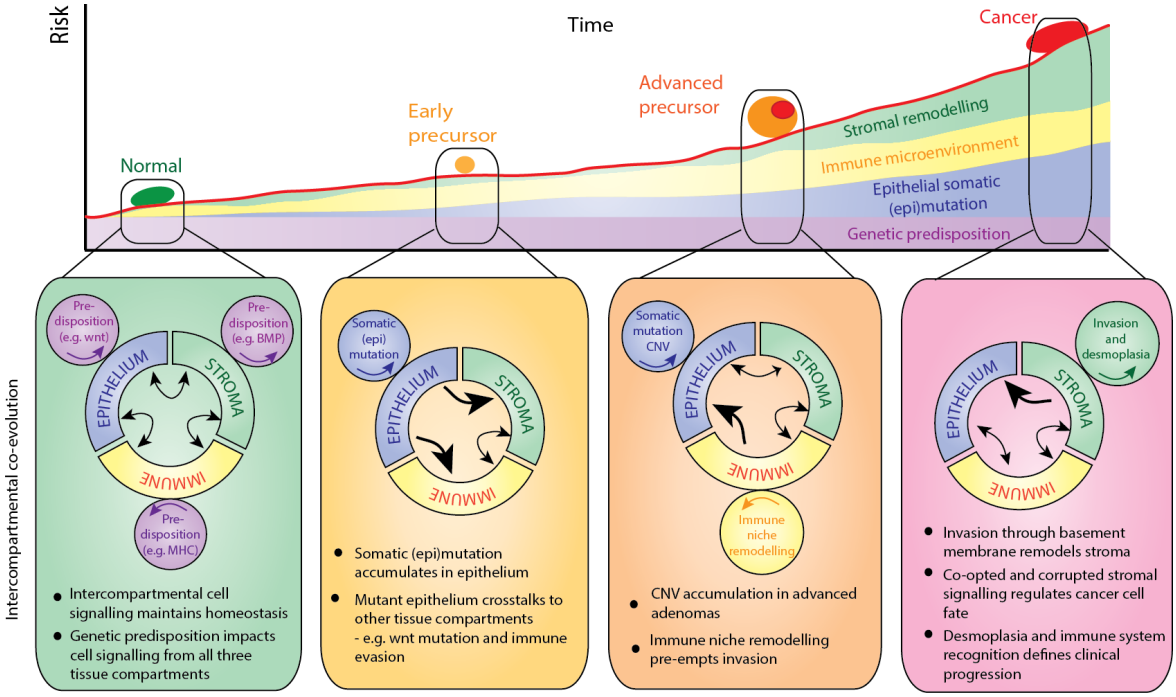

Figure 1. Co-evolution and interaction of epithelial, immune and stromal cell compartments defines temporal progression from normal through early and advanced precursors to cancer. Understanding this tri-compartmental interaction and how cell-to cell 'rules of engagement' evolve through tumour development is central to unravelling cancer cell heterogeneity and identifying new therapeutic targets.

Our aim is to use existing and emerging multiplex imaging technologies to examine inter-compartmental cell interaction (cell ecology) from normal intestine through to metastatic colorectal cancer (figure 1). Specific projects that would contribute to the students DPhil include:

1. Linking epithelial cell molecular classification with 'on-slide' morphological change. We have recently demonstrated the feasibility of correlating genomic molecular stratification classifiers with detectable morphological change on H&E slide alone. By undertaking machine learning digital image analysis in tumours with known molecular subtype classification, we can predict tumour subtype from the spatial analysis of epithelial, stromal and immune cells (AUC 0.82-0.88) and provide ways to analyse previously unclassified cases (13%) (Koelzer, Rittscher et al, under review). We now wish to develop this analysis to see if epithelial cell mutation and signaling disruption can result in 'on-slide' identifiable changes in tumour cell morphology. We will use machine learning analysis of digital pathology images of tumour epithelium in a large cohort of tumours stratified into colorectal cancer cell-intrinsic molecular (CRIS) subtypes. If we can show that specific molecular disruption correlates with definable tumour epithelial cell phenotypes, this will unlock the power of image analysis in CRC assessment. This work has huge clinical relevance; molecular subtypes are prognostic in CRC, and classification by image analysis can predict which patients need to be prioritized for expensive genomic analysis, effectively accelerating the adoption of molecular testing.

2. Stromal cell evolution at invasion. In colorectal cancer, tumour immune cell infiltration, detected by established multi-colour IHC panels, carries prognostic significance (2), and immune cell exclusion is associated with tumour emergence in carcinoma-in-adenoma tissue (Leedham and others, submitted). No comparative phenotypic analysis has been undertaken for the stromal cell compartment, which comprehensively changes at the point of invasion. We hypothesise that these important cells are vital in supporting the epithelial cell population and marshalling the tumour immune response. Oxford has led the way in defining stromal cell functional heterogeneity (3, 5). We will develop a panel of IHC/ISH markers to identify different intestinal stromal cell populations in CRC and explore stromal and immune cell ecology in carcinoma-in-adenoma samples which contain both invasive and benign tumour components.

3. Cell ecology in metastasis. Tumour cells pass through an important evolutionary bottleneck at metastasis, as they develop the capacity to disseminate, engraft and remodel a distant organ microenvironment. Interestingly, one of the earliest changes in early lymph node or liver metastases is the recruitment or transdifferentiation of stromal cells to support the engrafted cancer cells. Furthermore, recent

animal work has shown that inhibition of innate immune cells can prevent metastasis (6). Together these data indicate the power of the microenvironment in mediating fatal tumour dissemination. We will use paired primary tumour and secondary organ sections to generate cellular maps of metastatic disease and try to understand how, and why, migrating cancer epithelial cells remodel distant organ environments.

References

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18. Transcriptomic analysis of clock genes in breast cancer – Prof. Ray[1,2,3]

Primary Supervisor: David Ray

Additional Supervisors: Simon Lord

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: Either a biomedical/biological, computer science, mathematical or engineering background.

Project Summary

Abstract
Circadian stress is associated with increased cancer risk and recent work in cancer models has shown that changes in clock gene expression can result in tumour progression and rewired metabolism. However, links between host metabolism and the tumour microenvironment and expression of clock genes is little understood, and there is no published patient derived transcriptomic data. This study will utilise genetic modification of clock genes in breast cancer models to investigate their role in regulating tumour metabolism. RNASeq of prospectively collected breast tumour samples will characterise how tumour clock gene expression may differ between different timepoints. In addition, the relationship between clock gene expression and patient obesity and also tumour metabolic and immune gene expression signatures will be explored using transcriptomic data from historic trial datasets in which tumour metabolism has already been extensively profiled.

Justification for support
Circadian mechanisms control the 24 hour rhythmic cycles that regulate most aspects of mammalian physiology, including energy metabolism and immunity. These oscillations are synchronized by external environmental cues such as light, temperature, and intake of food, and a number of 'clock' genes regulate these processes. Large scale epidemiology and experimental studies link shift work, a stressor of the circadian system, to risk of cancer, with the strongest evidence applying to breast cancer. Shift work has also been associated with changes in the epigenetic landscape with particular reference to circadian loci. However, the precise mechanisms at play here are not known although genetic mouse models with mutations in clock genes (e.g. $Per2^{m/m}$) have been shown to predispose to lymphoma and hepatocellular carcinoma. Circadian clocks are understood to play a key role in regulating glucose metabolism, insulin sensitivity and lipid metabolism. Circadian misalignment, such as that due to shift work, is associated with reduced glucose tolerance and several SNPs in human clock genes have been shown to contribute to genetic susceptibility to obesity and type 2 diabetes. In obese patients the circadian clock genes have altered expression with loss of amplitude in oscillation; which likely impairs the coupling of the circadian clock to downstream genes and pathways. The oncogene c-Myc has recently been shown to disrupt the regulation of a number of circadian genes and ectopic myc expression altered oscillation of glucose metabolism and glutaminolysis in cultured cancer cell lines (Altman et al, Cell Metab, 2015). Another study of genetically modified mice prone to tumorigenesis with activated Kras and inactivated (p53 $Kras^{LSL\text{-}G12D/+}$;$p53^{fl/fl}$) in which the clock genes Per2 and BMAL1 were also ablated resulted in elevated consumption of glucose and glutamine and excretion of lactate compared to $Kras^{LSL\text{-}G12D/+}$;$p53^{fl/fl}$ mice (Papagiannakopoulos et al, Cell Metab, 2016). Recent work in mouse models has suggested that lung tumours have potential to rewire the hepatic circadian transcriptome (Masri et al, Cell, 2016).
However, overall, how host metabolism and the tumour microenvironment may link to expression of clock genes in human tumours is little understood, and there is no published patient derived transcriptomic data. We hypothesise that clock gene expression and hence regulation of circadian rhythm in breast cancer is altered by the tumour microenvironment and obesity. This may have implications for timing of therapeutic interventions on a 24-hour clock and also identify novel chronopharmacological targets in cancer.

This project brings together interests from cross departmental research groups: David Ray (Professor of Endocrinology, OCDEM), Simon Lord (Oncology).

Research objectives:

1) Nutrient supply the microenvironment and clock gene expression in breast cancer cell lines

Breast cancer cell lines will be cultured with differing nutrient concentrations (glucose, amino acids and lipids) and with and without hypoxia. Circadian function will be tested by tracking expression of virally-delivered circadian reporter genes (PER2-Luc, which allow oscillations to be tracked in real-time through several circadian cycles. This analysis will be supplemented by manipulation of specific core clock genes using CRISPR, and pharmacological approaches (targeting cryptochromes with a stabilising compound). The impact of endogenous circadian phase, or manipulation of the clock, on cellular bioenergetics will be tracked by using mass spectrometry techniques to identify differences in utilisation of glucose and glutamine as carbon substrates for lipid and nucleotide synthesis.

2) Metabolic imaging signatures and expression of clock genes

We have Poly A RNASeq data from two clinical trials (NEOMET and FRONTIER) in which PET-CT uptake of the radiolabelled glucose analogue, 18F-fluorodeoxyglucose and the synthetic amino acid tracer, 18F-fluciclovine was matched to breast cancer transcriptomic data. Here, we shall identify how these surrogate markers of anabolic metabolism match to tumour circadian clock gene expression. Additionally, we have RNASeq data from 30 (paired) samples taken from areas of high and low pH/hypoxia in 15 patients with glioblastoma that were imaged with Amide-CEST MRI (IMAGO trial) and this cohort will allow the student to explore how the microenvironment may link to clock gene expression. There are new computational tools (including CYCLOPS; Hogenesch lab), which permit virtual circadian series reconstruction, based on relative expression of the core circadian genes. Using these approaches we will estimate internal desynchrony within tumour samples, and relate circadian phase to genes expression, thereby identifying cycling genes in these cancers, for the first time.

3) Clock gene expression and metabolic pathway expression

Here using open access databases (TGCA) we shall build on this work to:

a) Link breast cancer transcriptomic metabolic pathway expression to clock gene signatures.
b) Use hypoxia metagene profiling to explore whether tumour clock gene expression is altered under low oxygen conditions in breast cancer
c) Correlate a proliferation signature to clock gene expression

4) Analysis of transcriptomic clock gene expression

We already have ethical approval for the collection of breast tumour samples for analysis of circadian gene expression patterns and routine sample collection (snap frozen at point of surgery prior to devascularisation) is already underway. Breast surgery occurs between 8am and 6pm in Oxford and the surgical team are confident that tumour samples taken at a variety of times will be available for assay in this study. Using RNASeq transcriptomic comparison between micro-dissected diagnostic biopsy samples and surgical samples taken at alternative times with allow paired analysis. In this ongoing project data is already being collected to characterise the patient host metabolic profile, including blood samples to measure insulin resistance, body composition (bioelectrical impedance) and BMI. By September 2020 we expect to have samples ready for sequencing from 100 patients (i.e. 200 paired samples) with associated timing of collection. Here, we shall assess how tumour clock gene transcriptomic expression may differ between different timepoints and how expression relates to metabolic and immune gene expression signatures. Additionally, this data will be integrated markers of host metabolism as above to understand the relationship between tumour clock gene expression and host obesity.

Translational potential:

The Ray group is focused on understanding how circadian mechanisms that control energy metabolism and immunity using wet laboratory and bioinformatic approaches. Simon Lord has an interest in cancer metabolism, obesity and the tumour microenvironment with reference to clinical drug/therapeutics development. Understanding how circadian mechanisms interact with the tumour micro- and macro-environment and also association with tumour anabolic metabolism and immune phenotype may allow the targeting of circadian mechanisms or modulation of treatment timing for therapeutic benefit.

19. Linking the microenvironment and tumour-associated macrophage infiltration and phenotype – Prof. Seymour[1,2]

Primary Supervisor: Len Seymour

Additional Supervisors: Kerry Fisher, Simon Lord

Eligibility: Track 1 and Track 2 only are eligible to apply for this project.

Required Background: Clinical trainee or medical undergraduate

Project Summary

Abstract
Tumour-associated macrophages typically represent a significant proportion of tumour mass and it has been shown that their interaction with tumour cells plays a role in modulating tumour behaviour. This project will investigate how tumour associated macrophage phenotype in breast cancer may alter dependent on nutrient abundance and in obese patients using, primary culture, genetic and metabolomic approaches. The work is directed toward understanding how the micro- and macro-environment may alter macrophage engraftment and behaviour with a view to macrophage based therapeutic approaches. The project will make use of patient samples from a range of patients with breast cancer.

Justification for support
Macrophages are a pleiotropic cell type, with functions ranging from providing one of the first barriers to infection to regulatory roles in growth and development. To encompass this broad range of activities, macrophages have been historically considered in two broad classifications; a classical "M1" type that functions mainly in inflammation, and an alternatively activated "M2" type with a broadly suppressive or regulatory phenotype. This designation aims to simplify a complex continuum of phenotypes, and it arose largely as an extension of the Th1 / Th2 paradigm. This situation is complicated further by the plasticity exhibited by macrophages, meaning that they have the potential to interconvert between phenotypes in response to changing environmental conditions. Tumour-associated macrophages (TAMs) sometimes representing as much 80% of the total tumour mass and they are thought to play many roles in supporting the malignant phenotype. As a consequence of this, several new drugs aim to interfere with TAM activities and thereby inhibit tumours indirectly.
M1 and M2 macrophages are understood to have distinct metabolic phenotypes. M1 macrophages display enhanced glycolysis but impaired OXPHOS metabolism whilst in contrast M2 macrophages have enhanced fatty acid oxidation and OXPHOS. Several studies have suggested that the abundance of glucose, glutamine or fatty acids may alter macrophage polarisation as well as obesity induced hypoxia. Obesity is associated with inflammation of adipose tissue and breast adipose tissue has been shown to contain more M2 versus M1 macrophages and this ratio increases in obese patients. Additionally, obesity breast samples have increased expression of genes associated with tumour-associated macrophages (Springer et al, Am J Pathol, 2019). Crown-like structures (microscopic foci of dying adipocytes surrounded by macrophages) are a histologic hallmark of an inflammatory process associated with obesity in postmenopausal women and associate with a higher risk of breast cancer and poor prognosis.
We hypothesise that the tumour microenvironment significantly alters macrophage uptake and phenotype into breast tumours and that this has implications for engraftment of autologous genetically modified macrophages within tumours. Ultimately this work is directed toward identifying markers that may determine levels of macrophage engraftment and hence therapeutic efficacy and possible treatment combinations to enhance this process.

Research objectives
1) Development of a macrophage/primary cell culture co-culture model in breast cancer

Initially breast cancer cell lines (Oestrogen receptor positive and triple negative) will be co-cultured with macrophages with differing levels of glucose, amino acids and lipids and under hypoxic and normoxic conditions to assess for changes in macrophage polarity using well described markers (iNOS, MMR). C13 tracing using labelled substrates and mass spectrometry will look to identify differences in utilisation of glucose and glutamine as carbon substrates for lipid and nucleotide synthesis between M1 and M2 macrophages. Further work will aim to develop a primary cell culture breast cancer model to recapitulate these experiments and allow assessment of tumour-associated macrophage metabolic phenotype in primary breast tumours from obese and non-obese patients.

2) Histologic assessment of macrophage infiltration between different breast cancer subtypes

Here we shall use CD68 staining on a panel of breast cancer samples (triple negative breast cancer; oestrogen receptor positive and HER2 negative; HER2 positive) obtained from the ORB breast cancer biobank to identify whether there are different levels of macrophage infiltration between different breast cancer subtypes. Additionally, we shall stain for Ca9 and markers of glycolysis (GLUT1) and amino acid uptake (ASCT2 and LAT1). Based on this work, using 80 biobanked samples from two clinical trials (NEOMET and FRONTIER) we may go on to assess whether PET-CT uptake of the radiolabelled glucose analogue, 18F-fluorodeoxyglucose and the synthetic amino acid tracer, 18F-fluciclovine correlate to macrophage infiltration.

3) Single cell sequencing of breast tumour macrophages in obese and non-obese postmenopausal women (collaboration with Kim Midwood)

Five obese patients and five of normal weight undergoing surgery for primary breast cancer will have tumour associated macrophages extracted from breast cancer samples (oestrogen receptor positive and HER2 negative). These will then undergo single cell RNA sequencing alongside matched 'normal' controls using macrophages extracted from biopsy material taken from the contralateral breast. Kim Midwood (Kennedy Institute) has already developed protocols for the single cell sequencing of macrophages and has expertise in analysing RNASeq data to understand the impact of the microenvironment on macrophage polarisation. Emphasis will be put on characterising metabolic pathways related to fatty acid oxidation, OXPHOS, glycolysis and amino acid metabolism and relating back to macrophage plasticity. Ethical approval is already in place for sample collection of primary tumour and from the contralateral breast in order to immunotype primary breast tumours.

Translational potential

The Seymour group is focused on targeted expression of transgenes using oncolytic viruses, and is exploring using monocytes as a potentially superior vector. Simon Lord has an interest in cancer metabolism, obesity and the tumour microenvironment with reference to clinical drug/therapeutics development. Understanding the consequences of the microenvironment on tumour-associated macrophage infiltration and phenotype will aid the development of these novel tools for targeted therapeutic delivery.

20. Investigating hypoxia and p53 pathway crosstalk in the cancer microenvironment – Prof. Lu[1,2,3]

Primary Supervisor: Xin Lu

Additional Supervisors: Sir Peter Ratcliffe

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: Either a biomedical/biological, computer science, mathematical or engineering background.

Project Summary

Abstract
Cancer cells don't grow in isolation; in the body they have complex interactions with the surrounding host tissues and immune cells. To understand more fully the molecular basis of tumorigenesis, cancer progression and responses to therapy we need to dissect the inter-relationships between the tumour and its environment. Hypoxia – low levels of oxygen – occurs in many tumour enviroments and can have a major impact on responses to cancer therapy. For example, hypoxic conditions can cause resistance to standard treatments such as radiotherapy. Thus, there are ongoing efforts to manipulate oxygen levels and cellular hypoxia responses to increase therapeutic efficacy. However, we need a better understanding of the crosstalk between hypoxia pathways, other key cancer signalling pathways and cells in the tumour microenvironment – particularly immune cells – to ultimately achieve better outcomes for patients. This project will harness the power of state-of-the-art technologies that provide high spatial and temporal resolution of gene expression and cellular signalling to dissect interactions between cancer cells and their environment. The focus will be on the crosstalk of hypoxia response pathways and signalling from the crucial tumour suppressor p53, particularly addressing how specific regulatory proteins influence the interplay between immune cells and tumour cells.

Research objectives and proposed outcomes
p53, the tumour suppressor known as the 'guardian of the genome', is inactivated by mutation or other repressive mechanisms in almost all human cancers. As a transcription factor, it regulates myriad cellular pathways and controls cellular life-or-death decisions. Similarly, the HIF factors are transcription factors that control numerous aspects of cellular and physiological behaviour in response to oxygen levels, which are often altered in tumours. Although we know that these two essential regulatory networks intersect, how do they act together in the tumour microenvironment? How does activation of one or both together influence tumour initation and progression? Are these influences cell autonomous or due to interactions with the immune system? This project will tackle these exciting questions using the latest strategies to resolve gene expression at high spatial and temporal resolution, such as single cell sequencing and highly multiplexed tissue imaging.
Prof Lu's group have recently made intriguing discoveries linking regulators of HIF with regulators of p53 and implicating immune cells in tumour suppressive effects via these pathways, providing a strong scientific foundation for this project. Also, in a cross-disciplinary collaboration with Hagan Bayley (Department of Chemistry), Xin Lu's group is developing new ex vivo 3D multi-cell type co-culture organoid models with realistic tissue architectures. These will be an innovative model for assessing the roles of hypoxia and p53 pathway regulators in cell-cell interactions, mimicking the tumour microenvironment.
Mentoring and training: Profs Xin Lu and Sir Peter Ratcliffe are internationally-renowned experts in p53 and hypoxia, respectively, so the student will be supported by ideal scientific leadership and expertise. Both supervisors have mentored numerous DPhil students, including Clinical Fellows, to successful graduation. As a Nobel Prize winning clinician scientist, Sir Peter Ratcliffe will provide an inspiration for the next generation of translational research leaders. Xin Lu has established collaborative networks throughout Oxford and beyond, and can enable the student access the infrastructure to make this project a success, including at the Ludwing and the Target Discovery Institute. The student will be given training in the necessary cell and molecular biology techniques including single cell genomics, 3D cellular co-culture and multiplex cell-resolution imaging

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

Translational potential of the project

The overarching aim of this project is to provide the molecular and cellular basis for enhancing treatment efficacy. Knowledge of the crosstalk between hypoxia and p53 signalling (which is triggered by current therapies) will feed into current clinical efforts to manipulate hypoxia to improve patient outcomes.

21. Investigation of tumour specific T cells in patients with glioblastoma to develop novel therapeutic strategies – Prof. Cerundolo[1,2,3]

Primary Supervisor: Vincenzo Cerundolo

Additional Supervisors: Puneet Plaha

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: A biomedical/biological or immunology background.

Project Summary

Abstract
Glioblastoma (GBM) is the most common and malignant primary brain tumour in adults. With full treatment the median survival for such patients is still only 14-24 months. The relentless and inevitable progression of GBM is thought to be facilitated in part by an immunosuppressive microenvironment, which weakens the ability of the immune system to mount an effective and tumour-eradicating response. This has formed the rationale for a novel line of research investigating immunotherapies for the treatment of primary brain tumours. Although there have been several immunotherapy based clinical trials conducted in recent years, as yet, no trial has shown any sustained long term benefit[1,2]. It was thought that this poor response due to a low mutational burden coexisting with a highly immunosuppressive microenvironment preventing the development of an adequate immune response against the tumour. However, more recent studies have shown that immunotherapies are able to generate an immune response in patients with GBM[3–5], supporting the need for further in-depth studies. Furthermore, we have shown that decitabine, a DNA-methyltransferase inhibitor, is capable of increasing expression of both a range of cancer testis antigens (CTA) and neoantigens (NAg). This increased in expression of immunogenic antigens is capable of inducing increased recognition and killing by cytotoxic T cells in a TCR-MHC restricted manner.
In this project we will interrogate the TCR repertoire within the tumour infiltrating lymphocyte (TIL) population at a single cell level to identify tumour specific T cells and their specificities. We will also assess the effect of a library of novel epigenetic modulating compounds on neoantigen presentation by tumour cells and subsequent immune recognition.

Aims

- Assess the TCR repertoire of TIL and peripheral blood.
    - Interrogate tumour reactivity and specificity of the TCR repertoire in patients
    - Identify clonally expanded TCR within patients
    - Identify shared TCR motifs across patients
    - Explore relationship between clonal expansion of TIL and clinical outcomes
- Explore the change in immunogenicity of tumours following treatment with novel epigenetic modulating compounds
    - Compound library screen of novel epigenetic modulating compounds
    - Interrogating changes in neoantigen expression in positive hits

Methodology

Formation of Tumour cell lines

We have recently optimised a pathway for derivation of patient derived GBM cell lines from primary GBM samples. We obtain fresh tumour samples from the Oxford Brain Bank, perform enzymatic digestion for form a single cell suspension. From this, single cells are then cultured in defined serum free media and passaged when confluent. We have derived FACS based system of characterisation of these tumour cell lines to confirm identity before experimentation. Utilising this method, we have been able to generate cell lines for over 20 patients.

Assessment of the TCR repertoire

We have developed and optimised a technique for indexed single cell TCR (scTCRseq) and paired transcriptome sequencing using a modified SMARTseq 2 protocol. In addition, we have further developed this technology to be able to transduce TCRs of interest into both reporter jurkat cell lines (GFP expression linked to NFAT promoter activity) and also primary T cells. These transduced reporter cells can then be used to interrogate the tumour specificity of the TCR repertoire against the autologous tumour line.
To complement this unbiased approach, we will also perform indexed single cell cloning of the TIL population as well as sub-populations of the peripheral T cells, using techniques well established in the laboratory[6]. We can then compare both the TCR repertoire of T cells that are able to be clonally expanded ex-vivo, as well as the tumour specific TCR, to the unbiased scTCRseq repertoire.
Screening novel epigenetic modulating drugs
We are establishing a collaboration with Prof. Bountra at the Oxford Structural Genomics Consortium to utilise their panel of novel epigenetic modulating compounds. We have a panel of potential NAg specific TCR for 4 patients, which we have transduced into reporter jurkat cells. These reporter cells will be used for our compound screen. We will treat the cell lines with and without the library of novel compounds and assess for increased jurkat activation following treatment. For the compounds that are the most effective, we will then perform whole transcriptome sequencing of primary tumour lines pre- and post-treatment to assess for upregulation of neo-antigen forming mutations. We will assess for changes in the epigenetic landscape specific to the mechanism of action of the compounds.

Translational potential
The results of this project will provide important insights into the existing T cell response within GBM. We will be able to both compare the changes in the TCR repertoire between patients as well as within the same patients at both primary diagnosis and recurrence, should they undergo further surgery. This information can be related to the clinical outcomes to look for any biomarkers of prognosis. Through the screening of novel epigenetic modulating compounds, we hope to be able to identify candidate compounds for further development into clinically viable drugs.

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22. Deciphering the role of TET2 mutation in determining lineage bias and cell fate decisions in Chronic Myelomonocytic Leukaemia – Dr. Quek[1,2,3]

Primary Supervisor: Lynn Quek

Additional Supervisors: Angela Hamblin, Skirmantas Kriaucionis, Chunxiao Song, Supat Thongjuea, Adam Mead

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: Candidates with a background in clinical medicine or biological sciences including biochemistry; or those with primary expertise in computation, mathematics or engineering who are interested to apply these to understanding cancer biology are also encouraged.

Project Summary

Abstract: Chronic myelomonocytic leukaemia (CMML) is characterised by proliferation of granulocyte-monocytic (GM) cells, bone marrow failure (anaemia, thrombocytopenia), and high risk of transformation to acute myeloid leukaemia (AML). It is incurable in most patients (median survival ~30 months), and is commonly driven by loss of function mutations in epigenome-regulating genes such as TET2 (~50% of CMML). Loss of TET2 function drives clonal dominance and GM lineage bias in haematopoietic stem-progenitor cells (HSPC) through mechanisms involving aberrant promoter hypermethylation, and altered chromatin function[1]. TET2-deficiency is correlated with loss of expression of putative tumour suppressors and promotes leukaemogenesis[2]. Proliferation of GM lineage cells occurs at the expense of megakaryocyte-erythroid (ME) cells, a finding correlated with anaemia and thrombocytopenia in patients[3]. We want to study how haematopoietic lineage fate is dysregulated in TET2-mutant (TET2m) CMML as this is likely to be the root cause of clinical disease which ultimately results in aggressive leukaemia. We will combine in vitro culture to characterise abnormal function in primary CMML HSPCs with detailed study of clonal and transcriptional heterogeneity in TET2m HSPC at the single cell level. We will study dysregulated gene expression, prioritizing putative regulators of haematopoietic differentiation pathways (e.g. transcription factors). We aim to identify epigenetic mechanisms that regulate cell lineage differentiation by investigating DNA cytosine (hydroxy)methylation and chromatin states. We will test if restoring normal methylation patterns using genome engineering can restore normal function to TET2m CMML HSPCs. Our overarching aim is to achieve a deeper understanding of epigenetic dysregulation in CMML, beyond the level of gene mutations, in the hope that this will widen the scope for new treatments in the future.

Background and Clinical Translational Importance: Poor clinical outcome in CMML is compounded by the lack of effective treatments. The only curative treatment is allogeneic stem cell transplantation, which is highly toxic and unsuitable for most patients. In CMML, the common myeloid progenitor (CMP), a progenitor that normally produces GM and MegE lineages, produce more GM progenitors (GMP) at the expense of ME progenitors (MEP) but the mechanism for this lineage bias is unknown[4]. TET2 mutations are acquired by either the stem or early progenitor cell early in disease evolution of CMML resulting in increased DNA and histone methylation[1]. Within this abnormal epigenomic landscape, late-acquired mutations in genes such as NRAS/ KRAS, CBL and RUNX1 further modulate disease phenotype (e.g. myeloproliferation). We do not know how TET2m-dependent epigenetic perturbations cause abnormal HSPC function in CMML. Each patient may have multiple clones that share a common TET2 mutation but differ in late-acquired mutations, giving rise to heterogeneity in the pathogenesis of CMML within an individual patient. Nonetheless, targeting TET2m-dependent aberrant epigenetic pathways shared by all disease subclones would be an attractive therapeutic strategy. Inhibitors of mutant isocitrate dehydrogenase (which indirectly promotes DNA and histone demethylation) or DNA hypomethylating agents have proved partially effective in some patients with myeloid malignancies. However, we need to understand the specific epigenetic mechanisms by which TET2m dysregulate HSPC function,

including the inherent clonal and epigenetic heterogeneity, to inform development of new, effective CMML treatments.

Preliminary data: We have access to ~40 clinically well-annotated TET2m CMML samples in the Oxford HaemBio biobank. We have immunophenotyped and flow-sorted HSPC populations in CMML samples. We have set up in vitro proliferation and differentiation assays to show that multipotent progenitors (e.g. CMP) in CMML exhibit marked GM-lineage skewing. Furthermore, we observed reduced erythroid progenitor output in patients which correlates with the severity of anaemia. We have used multi-genomic approaches to study primary haematopoietic cells both in bulk populations and in single cells in other myeloid cancers including AML and myeloproliferative neoplasms (MPN)[5,6]. The Mead lab have developed TARGETseq and have proven the ability to study clonal and transcriptional heterogeneity simultaneously in MPN [6]. The Quek lab have studied drug-induced differentiation of AML progenitors using Chromium 10x single cell RNAseq to find transcription factor-regulated pathways (manuscript in preparation). To study DNA methylation and hydroxymethylation, we are collaborating with the Kriaucionis and Song groups (Ludwig Institute Oxford) to perform TET-assisted pyridine Sequencing (TAPS)[7]. This novel technique avoids DNA degradation, and we have optimised it small numbers (~500-1000) of primary haematological cells. We have also optimised Assay of Transposase-Accessible Sequencing (ATAC-seq) to examine chromatin states in small numbers of AML cells (down to 500 cells). Together with our collaborators, we have the computational expertise required to analyse the experimental data (see Appendix 2 for analysis tools).

Project plan: Work package 1: From our bank of TET2m CMML patient samples (HaemBio biobank), we will select ~6 samples with adequate banked material for detailed experimental work. We will elucidate their clonal structures by mutational profiling in bulk cells using a myeloid genotyping panel followed by single cell genotyping. CMML HSPCs will be flow-purified and assessed in vitro to characterise lineage bias and differentiation arrest. Normal age-matched bone marrow (NBM) will be used as controls. We will genotype flow-purified haematopoietic populations at different stages of differentiation and lineage (HSPCs, neutrophils, monocytes, erythroid precursors) to establish the extent of lineage bias and differentiation arrest in TET2m subclones.
Work package 2: Using TARGET-seq, we will discover differences in gene expression in TET2m subclones of CMML HSPCs. Residual TET2 wildtype HSPCs in the CMML sample will act as internal controls. We may find differences in gene expression profiles of TET2m HSPCs versus wild type controls, and between different TET2m subclones which differ in late-mutations. Since the transcriptome reflects epigenetic changes, this work will demonstrate the link between clonal diversity and epigenomic heterogeneity within individual patients. In order to study the gene expression profiles of large number of CMML cells, we will perform high-throughput single cell RNAseq (scRNAseq) using the Chromium 10x platform. We will then cross-reference TARGETseq and 10x scRNAseq data to classify clonally-related cells by distinct gene expression signatures. 10x data enables us to examine cell states as a continuum of differentiation without the bias of flow sorting. We will perform trajectory analyses in CMML samples compared with NBM to see where lineage bias and/ or differentiation arrest has occurred. We will analyse gene expression profiles of TET2m CMML progenitors versus controls using co-expression models and promoter motif analysis to see if differentially expressed gene networks are regulated by a transcription factor.
Work package 3: Having identified transcription factors that may be involved in progenitor dysfunction in TET2m CMML, we will perform enhanced reduced representation methylation sequencing (eRRMethylSeq), using TAPS to generate libraries that discriminate between unmethylated cytosine, 5-methylcytosine and 5-hydroxymethylcytosine. We will look for differential methylation between TET2m progenitors and normal controls, focusing on promoters and putative enhancers (identified from the ENCODE database), to see if this may be the mechanism of altered gene expression. To ascertain the role of TET2 in modulating of gene expression via chromatin accessibility, we will perform ATAC-seq (as a proxy measure of active or repressive histone marks). We aim to identify regulatory elements that are vital for pathogenesis of TET2m CMML. We will validate this functionally by performing guide RNA-directed demethylation using a dead-Cas9/ TET-catalytic domain construct of putative regulatory elements in either primary CMML HSPCs or relevant cell lines.

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23. Modelling cancer stem cell dormancy using organoids and advanced 3D culture models – Dr. Boccellato[1,2,3]

Primary Supervisor: Francesco Boccellato

Additional Supervisors: Ahmed Ahmed, Colin Goding

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: A biomedical/biological background.

Project Summary

Abstract
Over recent years substantial advances have been made in our understanding of cancer and the development of a range of more effective therapies. Nevertheless, after apparently successful anti-cancer therapy, disease may recur even after many years owing to the presence of therapy-resistant cells. One of the principle causes of relapse is cancer cell dormancy. Some cancer cells stop dividing and enter a dormant state resembling that used by many physiological stem cells that divide to regenerate damaged tissue or replace cells that are naturally turned over. Why and how cells enter or emerge from a state of dormancy is unclear, but understanding how cells become dormant may offer opportunities for therapies designed to reduce relapse. Investigating human dormant cancer stem cells has been difficult due to the lack of appropriate *in vitro* models, and our current knowledge is extrapolated from experiments in mouse models or steady state analysis of human tumour masses. In this project we aim to use a newly identified biomarker for dormancy to detect and study this cell state *ex vivo* by using using patient-derived xenografts. Further we will use organoids and other advanced cell culture models (the mucosoids) to identify and isolate dormant cells in vitro. The specific aims are to: 1. Characterise the hallmarks of dormant cells; 2. Decipher the signals regulating the generation, maintenance and elimination of dormant cancer cells. This study promises to identify potential therapeutic vulnerabilities in cancer dormancy. To achieve this we will use approaches including single cell RNA-seq, label retention and lineage tracing, 3D culture systems, and live cell fluorescent reporter assays to compare dormant cancer cells with their physiological counterparts.

Research objectives and proposed outcomes
Tumours contain two kinds of non-proliferating cancer cells that are either quiescent or dormant. Most studies on dormancy do not distinguish between these two states. By examining physiological stem cells we have found that a hallmark of dormant, but not quiescent, cells is that they turn off expression of the majority of genes by shutting down transcription, evidenced by absence of an RNA polymerase II (PolII) modification associated with active transcription (Figure 1A). Since low transcription would reduce protein translation and suppress cellular metabolic activity, transcriptional silencing would allow cells to remain inactive for long periods. Importantly, such 'PolII-negative' cells are also found in human melanomas, mouse patient derived xenografts (PDX) (Figure 1B) and in 3D models of melanoma (not shown), but are not observed in 2D culture. The Goding lab has also developed a novel lentivirus-delivered ratiometric fluorescent reporter designed to enable detection of dormant PolII-low cells (Figure 1C). Using this reporter in intestinal organoids revealed that PolII-low cells at the base of the crypt (Figure 1C, open arrows) exhibited both red and green fluorescence, whereas PolII high cells (eg. white closed arrows) were red only. Although still to be tested widely, this reporter potentially enables live dormant cells to be isolated and characterised. The Boccellato lab has developed a homeostatic, long-lived (>1 year) stem cell based human primary cell culture, called

mucosoids, for the stomach (Figure 2A,B) and is currently using this technology to cultivate cells from fallopian tubes (Figure 2C,D).

Objectives

Characterise the hallmarks of dormant cells. Since PolII-low dormant cells can readily be detected in organoids, 3D culture and in tumours we will use the PolII marker in co-immunofluorescence assays to identify characteristics of dormant cells together with antibodies directed against candidate markers of interest. These include epigenetic marks, cell surface markers associated with minimal residual disease (eg aquaporins, CD36), and markers of stem cell populations identified by lineage tracing experiments in gastric and other organoid systems. In parallel, we will undertake laser-capture microdissection of human and PDX model tumour sections followed by RNA-seq to identify gene expression patterns associated with the PolII-low population, and by using the live cell fluorescent reporter assay in organoids or mucosoids, isolate candidate dormant cells for single cell-RNA-seq. We anticipate that we will identify biomarkers of dormant cells that will be useful for monitoring their generation and response to microenvironmental cues and to therapies, and a gene expression program that will reveal mechanisms underpinning the generation and maintenance of dormant cells. *B. Decipher the signals regulating dormancy*. Using the biomarkers identified, including low PolII, we will then vary culture conditions for physiological and cancer organoids and 2D cultures to identify signalling pathways that increase or decrease the proportion of dormant cells. These conditions include hypoxia, nutrient limitation, infection and signalling molecules such as TGF or TNF that are important in generating physiological stem cells, as well as targeted and chemotherapies. RNA-seq data will be mined for clues to key signalling pathways that may represent targetable vulnerabilities in dormant cells. The results obtained will generate novel insights into the origins of cancer stem cells, their relationship to physiological stem cells and should identify potential therapeutic vulnerabilities in dormant cells.

Collaborations

The project brings together three laboratories (one clinical, two academic) that have not previously collaborated to address a key outstanding question in cancer biology, namely can we identify a therapeutic vulnerability in cancer cell dormancy. The preliminary work is based on observations from the Goding lab, which has a long-standing interest in how the microenvironment generates phenotypic heterogeneity in cancer (Garcia Jimenez and Goding, 2019), primarily using melanoma as a model (Rambow et al , 2019). They recently identified PolII-low stem cells in multiple tissues, including in melanomas grown in 3D and PDX models, and have generated of a potential fluorescent reporter for dormancy. Ahmed's lab has recently identified 4 different cell populations, including stem cells, in the fallopian tube using single cell RNA-seq, and has shown that these populations are also found in ovarian cancer (Hu et al 2019). The Boccellato lab is new to

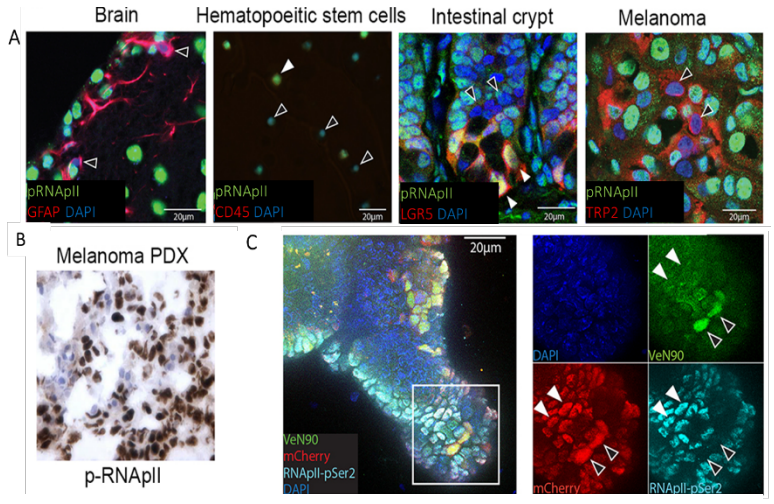

Figure 1. Detecting PolII-low dormant cells. A. Immunofluorescence using PolII-specific antibody (Green) together with lineage markers (red) and DNA (DAPI, blue). B. Detection of PolII-low cells in a melanoma PDX mouse tumour. C. Activity of a ratiometric fluorescent reporter to detect dormant cells. PolII-Low cells (open arrows) marked by absence of blue fluorescence are Green and red, but PolII-High cells are red only.

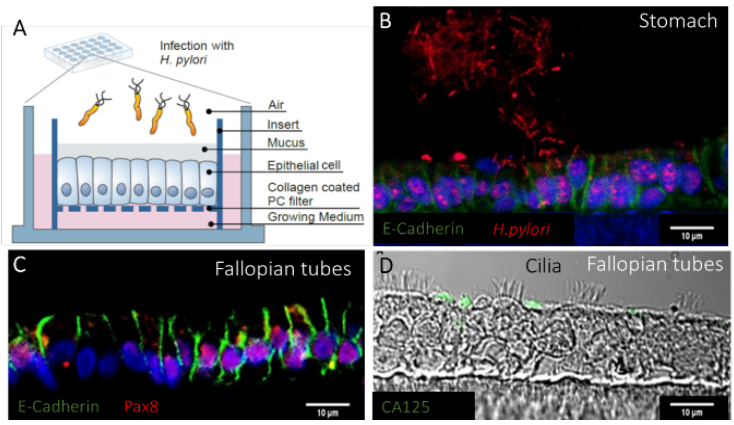

Figure 2. A. Schematic of a mucosoid culture. B. Gastric mucosoid culture infected with H. pylori. C. A fallopian tube mucosoid culture stained for the stem cell marker Pax8. D. the same culture stained for CA125 and with visible cilia

Oxford, but has developed a unique stem cell-dependent mucosoid culture system that accurately recapitulates the multi-lineage, highly polarized gastric epithelium that can be used as an in vitro model for gastric cancer (Boccellato et al 2018). The strength of this proposal comes from the shared interests and complementary expertise of each group in comparing physiological stem cells to those present within cancers, and especially in using the low-PolII status of dormant cells to identify and characterize dormant cells and their relationship to minimal residual disease.

Translational potential of the project - The project addresses a critical question in cancer using novel technologies and preliminary observations: what is the nature of cancer cell dormancy? We anticipate that the characterization of dormant cells may enable therapeutic vulnerabilities to be identified, with the potential to prevent and eradicate dormant cancer cells.

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24. Effects of ADT on combined RT and VTP treatment of pre-clinical PCa models – Dr. Bryant[1,2,3]

Primary Supervisor: Richard Bryant

Additional Supervisors: Ian Mills, Jens Rittscher, Freddie Hamdy

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: A biomedical/biological background.

Project Summary

Abstract:
Radiotherapy (RT) with concomitant androgen deprivation therapy (ADT) is a widely used standard of care for patients with high risk localised or locally advanced prostate cancer (PCa). Whilst this treatment is often successful, it can have significant long-term side effects, and a third of patients develop recurrence with limited treatment options. Vascular-targeted photodynamic therapy (VTP) is a novel minimally invasive surgical technique which can focally ablate PCa with high precision. VTP can treat low-risk low-volume PCa with minimal side effects, but it has not been used in combination with other treatments such as RT or ADT. This research aims to test the effects of ADT on combined RT and VTP treatment of pre-clinical PCa models

The **Bryant** laboratory is investigating the potential for additive/synergistic effects by combining RT and VTP in pre-clinical immunocompetent murine models of PCa. Initial results have established that sub-lethal doses of RT can have pro- and anti-tumorigenic immune responses within the tumour microenvironment, and can alter the function and structure of the tumour microvasculature. These initial experiments have characterised the necessary schedules and doses of RT and VTP for tumour control, and identified the sub-lethal treatment conditions for these immunological and vascular changes. Experiments combining RT and VTP in these pre-clinical models are on-going, testing the hypothesis that these two treatments may synergise, such that the dose of RT and VTP needed for complete tumour control/cure might be reduced. Such an observation would lead to early phase clinical trials in patients, with the potential to improve clinical practice by reducing RT-related side effects and increasing the cure rate of PCa. However, to date these pre-clinical experiments have not incorporated ADT. Given that ADT plus RT is a standard-of-care for high-risk localised and locally advanced PCa, it is necessary to investigate whether administration of ADT plus RT modulates any additive/synergistic effects of VTP. ADT modulates the tumour immune microenvironment (1-3), and in addition may initially reduce PCa tumour microvascularity and increase hypoxia (4-8) prior to a later revascularisation and reoxygenation phase. This initial PCa tumour vessel degeneration post-ADT may be mediated by dysfunction of androgen-sensitive endothelial cells. Other evidence suggests that ADT results in an improved and highly functional vascularisation of PCa tumours one month post initiation of ADT (9-10), which may be driven by initial hypoxia, stabilisation of HIF-1α, expression of HIF-1α target genes including VEGF, and acquisition of alternative mechanisms for androgen-sensitive endothelial cells to reproduce the vascular network. Therefore, it is possible that concomitant ADT influences any beneficial effects of combining RT and VTP, i.e. it may be that combining RT and VT may be best done around one month after commencing ADT, rather than immediately after ADT. It may also be that ADT prior to VTP may obviate the need for RT to induce neovascularisation. This hypothesis requires testing in our established pre-clinical models, as it would have important implications for the design of early phase clinical trials. In conclusion, this research proposal aims to test the effects of ADT on combined RT and VTP treatment of pre-clinical PCa models.

Translational potential of the project:
Given that ADT plus RT is a standard of care treatment for localised and locally advanced PCa, and given that VTP has already been shown to be a safe and efficacious treatment of low-risk low-volume PCa, combining these treatments with the correct timing and sequence is eminently feasible, although this needs to be informed by pre-clinical results. An early phase clinical trial of these treatment combinations could be in progress within a

short timescale (~3 years, though outside of the remit of this DPhil proposal). Such a trial, if successful, could lead to a phase 3 randomised clinical trial investigating this combined treatment approach against standard of care ADT and RT in high risk localised / locally advanced PCa. Such a trial has the potential to change practice by reducing toxicity and improving outcomes for patients with this common malignancy. It also has translational potential for other cancer types, including lung, breast, pancreas and colorectal cancer.

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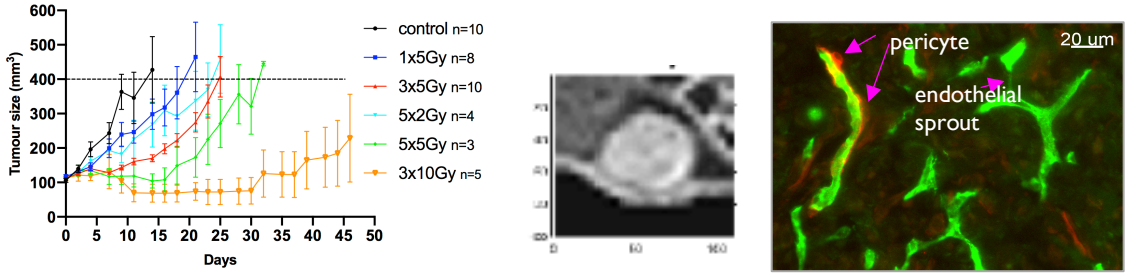

Figure 1. **A)** *In vivo* tumour growth delay of flank PCa tumour allografts post-RT; **B)** DCE-MRI imaging of flank PCa tumour allografts; **C)** Multiplex immunofluorescence analysis of tumour vasculature.

25. Understanding the therapeutic efficacy of PD-1 blockade in pancreatic cancer – Dr. Sivakumar[1,2,3]

Primary Supervisor: Shivan Sivakumar

Additional Supervisors: Michael Dustin, Mark Middleton

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: A biomedical/biological background.

Project Summary

Abstract of the project
Pancreatic cancer has the worst survival of any human cancer. Breakthroughs in treating this disease have been modest. Checkpoint immunotherapy has had significant impact in several epithelial tumour types. Phase 2 and 3 trials in pancreatic cancer with checkpoint immunotherapy have all been negative but within the trial data, 5-10% of patients have a response. As we move into the era of personalised medicine, it is important to look at who these responders are and to find biomarkers of response. This project will be performed in two parts. We will be running a small experimental trial where patients with pancreatic cancer will receive either pembroluzimab (anti-PD1 antibody) or pembroluzimab and chemotherapy. All patients will have pre- and post-treatment tissue for transcriptomic analysis and molecular imaging. Further bloods will be taken for immunophenotyping and circulating tumour DNA for analysis of response and biomarker stratification. The second part of the project will require the student to treat pancreatic cancer with pembroluzimab in an ex vivo setting, dissociate the tissue and perform single cell sequencing to understand the single cell changes. The fellow's project will be to elucidate the biology of response to anti-PD1 therapy in pancreatic cancer using the clinical sample material collected.

Justification for support
Pancreatic cancer (PDAC) has the worst prognosis of any cancer[1]. Our group has previously shown that PDAC has distinct transcriptional immunophenotypes[2]. We have now started to characterise the tumour immune microenvironment in single cell detail. We have shown there is some PD-1 activity on CD4 and CD8 effector T cells in PDAC (unpublished). Clinical trials for checkpoint inhibition in pancreatic cancer have been negative so far but we know within the data approximately 5-10% of patients respond to anti-PD1[3,4]. The study and treatment of pancreatic cancer is a priority for the Oxford cancer centre. This DPhil represents our first attempt at early translation of our work. The project is split into two parts: 1) Analysis of clinical trial material 2) Analysis of the effects of PD-1 on pancreatic cancer cells at a single cell level. Our clinical trial platform is called OXPANC, which is designed to test drugs that can perturb the microenvironment of the tumour in the metastatic setting. The trial will consist of dosing 20 patients with single agent pembroluzimab and 20 patients with pembroluzimab and chemotherapy. Biopsies are taken of the primary pancreatic cancer at the start and at the end of treatment with serial blood sampling during the course of treatment. Furthermore, we have a lot of expertise studying pancreatic cancer at a deep single cell level to study the epithelial cells, immune cells and stromal cells. We have developed a significant resource of single cells of pancreatic cancer, which are treatment naïve and treated with chemotherapy. The student will obtain ten pancreatic cancers after they have been resected from patients and treated in an ex vivo setting with pembroluzimab. The fellow will dissociate and sort cells into tumour, immune and stromal cells and single cell sequence. They will then use bioinformatics techniques to understand the changes in the single cell transcriptome and cell-cell interactions. They will use previous datasets we have generated as control groups for their analysis.
The candidate will be based in two world-class departments (Oncology and KIR) in Oxford but is expected to liaise with many clinical departments and other university departments. The fellow will be mentored by Dr Sivakumar, Professor Middleton and Professor Dustin who have in combination developed significant expertise in studying the tumour microenvironment of PDAC and have expertise in translational trials.

Research objectives and proposed outcomes

Objectives:

- Describe intra-tumoural transcriptomic changes before and after single agent anti-PD1 and combination anti-PD1 and chemotherapy.
- Ascertain whether white cell IHC (including CD8, CD4, Ki67, FoxP3 and PD-L1 T cell) are predictive of response
- Describe T cell phenotypes in periphery and how these respond to immunotherapy
- Does circulating tumour DNA reflect treatment response to immunotherapy in pancreatic cancer
- To understand the effects of PD-1 on single cell transcriptomes
- To understand the effects of PD-1 on cell-cell interactions

The proposed outcomes is to analyse the clinical trial material that is collected, develop a single cell resource that has been treated with PD-1 and to write up the findings for a manuscript. The applicant will be trained in fundamental immunology, in depth analysis of samples from human tissue with techniques such as flow cytometry, single cells sequencing CyTOF, immunofluorescence as well as transcriptomic and image analysis. They will also appreciate how to frame clinical problems as tractable research questions and translational medicine experimental design. The fellow would also be expected to present the results in national and international meetings. The findings from the study would inform us how to direct further PD1 immunotherapy in this disease setting.

The research itself is highly novel and urgently needed in a cancer of unmet need. This project will show if there is a biological rational of using PD-1 therapy in pancreatic cancer. The project is a highly collaborative project with the student needing to interface with many departments in the hospital and within the university. Expertise in genetics will be learned from the genomics centre in the Wellcome Trust Centre for human genetics, imaging from the translational pathology laboratory in the university oncology department, CyTOF from the Botnar and circulating tumour DNA with colleagues in the Ludwig institute.

Translational potential of the project

This work will help identify which subset of pancreatic cancer patients benefit from anti-PD1 therapy and help us understand how we could augment the agent to gain a better response. This study will give us a real insight into which patients respond and which do not and why this is the case. We envisage that if there is a clear group of responders, we can develop a stratification system so this can feed into national trial platforms such as Precisionpanc to guide how we should treat PD-1 responsive patients on the national arena.

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26. The molecular mechanisms underlying the roles of CDK12 and CDK13 in cancer – Prof. Murphy[1,2,3]

Primary Supervisor: Shona Murphy

Additional Supervisors: Chris Norbury

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: A biomedical/biological background.

Project Summary

Abstract

Cyclin-dependent kinases (CDKs) and their activating cyclin partners regulate a variety of critical cellular processes, including progression through the cell cycle (1) and inhibitors of these kinases represent a valuable new class of anti-cancer drugs (2). Some members of the CDK family are responsible for the differential phosphorylation of the C-terminal domain (CTD) of RNA polymerase (pol) II during the transcription cycle (3). In humans, the CTD comprises 52 repeats of the consensus heptapeptide Tyr1/Ser2/Pro3/Thr4/Ser5/Pro6/Ser7. The closely related kinases CDK12 and CDK13 play important roles in CTD phosphorylation (3). The activation of transcription elongation by CDK12 is required for an efficient DNA damage response (4,5,6,7), defects in which can contribute to the development of a wide variety of cancers. However, the role of CDK13 in the regulation of gene expression and how dysregulation of this kinase contributes to tumorigenesis is largely unexplored. Importantly, inhibition of CDK12 is synergistic with DNA-damaging chemotherapy or PARP inhibitor treatment and compounds that inhibit both CDK12 and CDK13 are currently in trial for treatment of, for example, triple-negative breast cancer (8). Elucidation of the molecular mechanisms regulated by CDK12 and CDK13 is therefore an important step towards the judicious development of drugs that target these kinases. In order to gain insight into the normal roles of CDK12 and CDK13 and how dysregulation of these kinases may lead to cancer, we have made human cell lines with ATP analogue-sensitive versions of endogenous CDK12 and/or CDK13, which allow 'chemical genetics' to be performed through rapid and specific inhibition of these kinases (9). This project aims to use these cell lines to identify the targets of CDK12 and CDK13 beyond the pol II CTD and to elucidate the roles of these kinases in expression of human protein-coding genes by analysing transcription and RNA processing genome-wide using state-of-the-art molecular techniques. As we have also found that other CTD kinases play roles in regulating the cell cycle (Murphy lab*, unpublished*), which is often dysregulated in cancer, the role of CDK12 and CDK13 in regulating the cell cycle will also be investigated.

Research objectives and proposed outcomes

CDK12 and CDK13 are very similar large kinases and both are found in complexes containing cyclin K (3). CDK12 dysregulation is mainly associated with cancers of the ovary, adrenal gland and haematopoietic/lymphoid tissues, whereas dysregulation of CDK13 is mainly associated with colorectal, oesophageal and central nervous system tumours (https://cancer.sanger.ac.uk/cosmic).

In the cell lines we have made with analogue-sensitive versions of endogenous CDK12 and/or CDK13, the large phenylalanine 'gatekeeper' residue near the active site is replaced by a much smaller glycine residue. This mutation renders the enzyme selectively sensitive to inhibition by commercially-available bulky adenine analogues (9). We have already carried out analysis of the effect of CDK12 inhibition on transcription and shown that inhibition of this kinase reduces the recruitment of key elongation factors and slows down elongation of transcription of human protein-coding genes, which has a disproportionate effect on the expression of long genes (10).

Preliminary analysis indicates that inhibition of CDK13 rather affects the amount of pol II that enters productive elongation, emphasising that the two kinases are not entirely redundant. However, we have found using our CDK12/13 double analogue-sensitive cell line that these two kinases are redundant for activation of some RNA processing events (Murphy lab *unpublished*). Due to the high homology of the active sites of CDK12 and CDK13, the small molecule drugs that are being developed to inhibit CDK12 also inhibit CDK13 (6,8), raising the

possibility of unwanted off-target effects. It is therefore important to understand the precise roles of these two kinases to judiciously design alternatives strategies to target pathways controlled by one or other of these kinases.

The aim of the project is to fully characterize the effect of inhibiting CDK12 and/or CDK13 on pol II CTD phosphorylation, transcription and RNA processing using genome-wide analyses including mNET-seq, RNA-seq and ChIP-seq (11). The student will also identify the primary targets of the kinases by phosphoproteomics before and after treatment of cells with the specific inhibitors. They will also determine the effect of loss of enzyme activity on cell biology, including the cell cycle, and characterize the underlying molecular interactions using state-of-the-art molecular and biochemical techniques. All necessary gene expression and cell biology analysis techniques, including genome-wide analysis, phosphor-proteomic and bioinformatic protocols are already in routine use in the Murphy laboratory and the student will therefore be trained in a wide range of molecular biology and biochemistry technology as part of a well-funded, internationally-leading research team. Chris Norbury will provide the necessary cell cycle expertise. The expected outcomes are the elucidation of the precise roles of CDK12 and CDK13 in gene expression and cell cycle regulation and the underlying molecular mechanisms. The student will be supervised on a daily basis by a postdoctoral fellow and Professor Murphy to learn all the necessary molecular techniques and bioinformatic analysis and will have regular strategy meetings with Professors Murphy and Norbury. The student will attend all appropriate courses and seminars in the Dunn School as part of the training.

The award will facilitate a new collaboration between the Murphy and Norbury laboratories to investigate the role of CDK12/13 in regulation of the cell cycle. This is an important aim as it will elucidate how dysregulation of these transcriptional kinases can promote tumorigenesis and how drugs that inhibit these kinases would impact the cell cycle of healthy cells.

Translational potential of the project

The outcomes could ultimately lead to the identification of CDK12/13 substrates that would be more specific targets for development of new cancer treatments. We are already in contact with our established chemistry collaborators Professors Chris Schofield and Angela Russell, and we envisage that the results of this project will provide information necessary for the judicious design of new small molecule drugs for cancer treatment.

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27. Vaccine development for prevention of Epstein Barr virus-associated cancers – Prof. Hill[1,2,3]

Primary Supervisor: Adrian VS Hill

Additional Supervisors: Simon J Draper, Alexander D Douglas

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: Either a biochemistry, pathology, immunology, microbiology or a related a background.

Project Summary

Abstract

EBV causes 1-2% of global cancers (principally lymphomas, nasopharyngeal and gastric carcinomas)- more than any other infection for which there is no vaccine or curative treatment. To our knowledge, there are currently no active clinical trials of prophylactic vaccines against EBV. There are good grounds to believe a vaccine is feasible: for example, there are effective vaccines against a related herpesvirus (varicella zoster virus, which causes chickenpox and shingles).

The student will develop and perform pre-clinical evaluation of candidate EBV vaccine immunogens. The focus will be upon the induction of virus-neutralising antibody, in line with increasing evidence that anti-EBV antibody can be protective (for example, acquisition of EBV by infants does not occur until after the loss of transplacentally-transferred maternal antibody). Antibodies elicited by immunisation of rodents with immunogens based upon the viral gB, gH/gL and gp350 antigens will be tested for their capacity to neutralise EBV *in vitro,* and their ability to protect against EBV challenge by passive transfer into immunodeficient mice engrafted with human B cells (it is not possible to infect immunocompetent mice with EBV). Antigens will be delivered using modern vaccine technologies which have been developed to tackle other immunologically challenging pathogens, including the use of Oxford's Spycatcher-Spytag virus-like particle technology (which can dramatically enhance immune responses, but has not yet been applied to anti-cancer vaccines). The mechanistic basis of neutralisation may be explored by isolation and characterisation of antigen-specific neutralising monoclonal antibodies (which might themselves have value as products for prophylaxis of EBV-driven disease in highly immunocompromised patients eg those susceptible to PTLD).

The project will also include an observational clinical study of salivary EBV shedding, and immune responses against the virus, in healthy carriers. This will complement the direct vaccine development work, and aims to pave the way for the use of salivary virus shedding as an endpoint in rapid small-group early-phase clinical studies. Vaccine developers targeting other pathogens commonly challenge informed and vaccinated human subjects with the target organism, but challenge of volunteers with an oncogenic virus which causes lifelong infection is clearly unethical. The few previous clinical trials of candidate EBV vaccine efficacy against virus acquisition have involved hundreds of volunteers being followed for years. There are however large numbers of healthy adults persistently infected with EBV, in whom salivary shedding of virus can be detected. Much like vaccination of healthy carriers of varicella zoster virus prevents virus reactivation (shingles), an effective EBV vaccine should suppress reactivation (epithelial infection leading to salivary shedding). Use of this as a trial endpoint would permit the efficacy of a vaccine to be evaluated in a few months in ~20 subjects. It may also be a biomarker of vaccine-induced reduction in nasopharyngeal cancer risk: EBV infection of the pre-malignant nasopharyngeal epithelium cell is a late event in oncogenesis, occurring *after* a series of prior mutations render the cell permissive, and suggesting that cancer may be preventable by vaccination of adults who are *already* carrying EBV. This work will build upon supportive preliminary data tracking salivary shedding by qPCR in a small cohort. The study will also provide the opportunity for a detailed analysis of relationships between immune responses and levels of virus shedding in healthy volunteers, which may be extended to make use of the large panel of saliva and serum samples available from the UK Biobank.

The project aligns with Centre objectives (infection and immunity, and prevention of gastric and haematological malignancies) and is based upon a collaborative approach between three supervisors, two of whom are new to the cancer field and will provide expertise in virology and antibody-inducing vaccine development. It will provide the student with outstanding training in immunology and translational / experimental medicine, relevant not only to a career in vaccinology but also to the broader fields of immuno-oncology and early-stage biological drug development.

RESEARCH OBJECTIVES & OUTCOMES

a) Pre-clinical proof-of concept of a prophylactic EBV vaccine, suitable to support an application for funding for GMP manufacture and Phase I trial. The primary aim is translational, but the aim will be to produce high-quality data suitable for high-impact publication. Novelty will come from the application of recently-developed technologies (VLP platforms, adjuvants, and immunogen expression systems) to overcome shortcomings in the quality and quantity of antibody induced by previous candidates.

b) Data adequate to permit the design of early-phase vaccine studies using salivary virus shedding as an endpoint to indicate vaccine-induced suppression of viral reactivation. This would be a major advance and would allow the field to evaluate many more candidate vaccines than has hitherto been possible; an iterative approach based upon many small trials has been central to the development of vaccines against other immunologically challenging chronic infections such as malaria.

c) Analysis of correlation between a panel of measured immunological responses and levels of salivary virus shedding. Previous efforts to relate salivary virus shedding to levels of immune responses have used very limited immunological analysis, have been substantially statistically underpowered, or have used single measures of shedding in an individual (failing to take account of the substantial fluctuation in levels of shedding over time). The student will be well positioned to overcome these limitations as they will be tracking a suitable cohort (aim B), producing high-quality conformationally-accurate recombinant proteins and performing virus neutralisation assays for the vaccination work, and will have access to the Jenner's outstanding expertise in characterisation of pathogen-specific T cell responses. The resulting insights will guide future vaccine development by suggesting the appropriate targets and nature of vaccine-induced immune responses.

TRANSLATIONAL POTENTIAL

The project aims directly at development of a novel cancer prevention tool. The burden of EBV-driven cancer (lifetime mortality 0.2-1% for >1 billion people in China, SE Asia, Africa) suggests a strong public health and economic case for an effective vaccine, and a large market to motivate late-stage development. The case for EBV vaccination in the UK may be boosted by the virus' likely contribution to autoimmune disease, as well as cancer.

The Jenner Institute is Europe's largest centre for translational vaccinology, having conducted >100 Phase I & II vaccine trials in recent years, and with a strong record of patent filings and spinout company generation. The DPhil will thus be well-suited to a student who wishes to pursue both academic excellence and real-world impact through true bench-to-clinic translation.

28. Targeting immunosuppression in hypoxic oesophageal cancer – Dr. Parkes[1,2,3]

Primary Supervisor: Eileen Parkes

Additional Supervisors: Ester Hammond

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: A biomedical/biological, computer science, mathematical or engineering background.

Project Summary

Abstract

Oesophageal adenocarcinoma (OAC) is increasing in incidence in the UK, and carries strikingly poor outcomes. There is therefore an urgent need for the discovery of novel interventions. One of the critical factors influencing response to therapy and patient outcomes is hypoxia, where hypoxic tumours are typically resistant to chemotherapy, radiotherapy and immunotherapy. Hypoxia is common in OAC and promotes an immunosuppressive tumour microenvironment.

A key mediator of immune responses is the cGAS-STING pathway, where cytosolic DNA results in cGAS-STING pathway activation and initially increased immune signalling. However, chronic cGAS activation results in recruitment of immuno-suppressive cells and shut-down of immune responses[1,2]. We propose a novel mechanism of immunosuppression in the context of hypoxia:

(1) Physiological hypoxia results in replication stress and chronic cGAS stimulation.
(2) Chronic cGAS signalling upregulates ENPP1 expression in the tumour microenvironment.
(3) ENPP1 promotes and maintains immunosuppression by the breakdown of 2'3'cGAMP[3] to adenosine *(Figure 1).*

Therefore, ENPP1 inhibition represents a novel approach to overcoming resistance to STING agonists and immunotherapy in oesophageal cancer.

This comprehensive approach will identify the role of ENPP1 and 2'3'cGAMP in hypoxia-mediated immunosuppression, use a novel inhibitor of ENPP1 in the context of hypoxia to identify innovative combination approaches and determine the role of hypoxia in response to STING agonists.

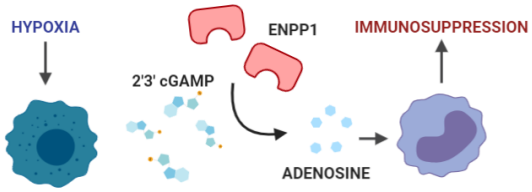

Figure 1: Hypoxia results in cGAS activation, and therefore extracellular 2'3'cGAMP. 2'3'cGAMP is hydrolysed by ENPP1 resulting in an adenosine-rich immunosuppressed micro-environment.

Objective (1) Assess the impact of hypoxia on ENPP1, 2'3' cGAMP and adenosine production in oesophageal cancer and immune cells.

We will test the model outlined in *Figure 1* using both 2D and 3D culturing of a well characterised bank of OAC cell lines available within the Hammond lab. Selected cGAS/STING-high and -low models will be used to determine the effects of acute, intermittent and chronic hypoxia on the generation of cytosolic DNA. Activation of cGAS/STING pathway will be identified using immunofluorescence, western blotting and chemokine analysis. ENPP1 production will be assessed in these conditions, as well as use of the Bioanalysis Small Research Facility to perform liquid chromatography/tandem mass spectrometry to measure 2'3'cGAMP and adenosine in these conditions. Similar assays measuring 2'3'cGAMP, ENPP1 and adenosine will be performed using models of macrophages, dendritic cells and cancer-associated fibroblasts. Therefore, a novel targetable pathway of

hypoxia-mediated immunosuppression will be carefully defined, with identification of cells within the tumour microenvironment responsible for the production and maintenance of ENPP1.
Outcomes: Identification of factors promoting production of extracellular 2'3'cGAMP and ENPP1. Quantify and characterise production of ENPP1 by cells found within the tumour microenvironment.

Objective (2) Identify the role of ENPP1 inhibition in hypoxia as single agent and combination therapy
ENPP1 inhibitors are now in preclinical development, with first-in-human studies planned shortly. However, the optimal context for this treatment has not been identified. As ENPP1 is upregulated in hypoxia, we hypothesise it has a pro-survival role in hypoxic tumours. We will therefore use the ENPP1 inhibitor STF-1084 (in house) in hypoxic conditions, determining impact on proliferation, invasion and migration will be determined. CRISPR-cas9 mediated ENPP1 knockout models will be developed. Treatment of these novel models with chemotherapy (5-FU, oxaliplatin and docetaxel) and radiotherapy will allow characterisation of ENPP1 inhibitors as a potential radiosensitiser. Treatment of immune models with STING agonist and ENPP1 inhibitor combination therapy in hypoxic and normoxic conditions will identify potential dual and triple therapy combination approaches with the potential to improve response to conventional and novel therapies in early phase clinical trials.
Outcomes: Characterisation of novel ENPP1 inhibitors in the context of hypoxia and in combination with conventional therapy.

Objective (3) Determine the effect of hypoxia on response to STING agonists.
STING agonists are now in clinical trials, opening in Oxford, given as single agents and in combination with immune checkpoint therapy, with a focus on biomarker discovery. There is therefore potential for collaboration with industry partners to gain access to gene expression data from these trials. We will determine if hypoxia is a potentially targetable mechanism of resistance to STING agonists, via production of ENPP1 or other means. Using commercially available and in-house STING agonists, we will treat macrophages and dendritic cells in the context of acute, intermittent and chronic hypoxia. Responses to STING agonists will be measured using chemokine analysis. Targetable mechanisms of STING agonist resistance will be identified.
Outcomes: Discovery of mechanisms of resistance to STING agonists. Potential combination approaches to improve response rates to STING agonists in the clinic.

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29. The role developmental stage specific programmes in infant/childhood leukaemia – Dr. Roy[1,2,3]

Primary Supervisors: Anindita Roy, Thomas Milne

Additional Supervisors: Philip Ancliff

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: A biomedical/biological background.

Project Summary

Abstract: There have been remarkable advances in the treatment of childhood acute lymphoblastic leukaemia (ALL) where mean survival rates are now >90%[1]. However some subtypes, such as infant ALL (iALL), which is defined by an onset before 12 months of age, have high relapse rates and poor survival[2]; and more effective therapeutic strategies are needed. Natural history and molecular studies indicate that all cases of iALL originate *in utero*[3], it is distinct from childhood ALL[4]; and most are caused by a balanced translocation of the *MLL* gene resulting in an oncogenic MLL fusion protein, most commonly MLL-AF4[5]. This single 'hit' seems sufficient to produce an early onset aggressive leukaemia. The identity of the target prenatal B progenitor cell(s) in iALL is unclear. We have recently defined unique prenatal B cell developmental pathways and a possible target cell for iALL[6]. These cells display a distinct, ontogeny-related gene expression pattern that is not seen in adult type progenitors, and they share many features with iALL cells. This project will explore whether these specific gene expression programmes provide a permissive cellular context for prenatal B-progenitor leukemia initiation, especially iALL, and whether they can be modified to develop new approaches to treatment.

Research objectives and proposed outcomes:

Hypothesis: Developmentally-regulated characteristics of lymphoid progenitors provide the molecular context for initiation and evolution of infant/childhood ALL.

Aim1: To define the site- and stage-specific gene expression programmes in normal prenatal and postnatal lymphoid progenitor cells
The gene expression and epigenetic profiles of human prenatal lymphoid progenitors will be compared with postnatal progenitors (from normal healthy children and adults) in order to identify unique prenatal gene expression patterns. A number of state-of-the art techniques will be used, including single cell Chromium 10x platform, bulk RNASequencing and ATAC-sequencing. The transcriptomes of prenatal and adult progenitor cells will also be compared with primary leukaemia cells (ALL) to identify shared gene expression networks likely to be important in leukaemia initiation.

Aim 2: To define specific molecular characteristics of prenatal lymphoid progenitors that initiate, maintain and propagate infant/childhood ALL
So far iALL has been difficult to model and limitations in these models may reflect the need for an appropriate prenatal cell context[7]. Novel *in vitro and in vivo* models of human ALL generated in our lab will be used to investigate how prenatal-specific gene expression programmes co-operate with iALL-associated oncogenes, such as MLL-AF4. This work will include using CRISPR-Cas9 mediated gene editing, which has already been established in our lab, to address the requirement of specific prenatal programmes in leukaemia initiation and maintenance.

Aim 3: To study the role of these developmental stage-specific characteristics in drug responsiveness of iALL and childhood leukaemia
Preliminary data suggests that prenatal gene expression patterns are more prevalent in iALL compared to paediatric ALL. Two approaches will be used to investigate the contribution of these gene expression

patterns to the treatment resistance of iALL cells, Firstly, previously published, as well as our own, RNAsequencing data from infant, paediatric and adult ALL patients will be analysed to investigate the correlation with treatment response and relapse. Secondly, model systems/gene editing and overexpression or knockdown of key prenatal specific genes will be used to investigate the impact of specific genes and/or pathways on ALL biology *in vitro* and *in vivo*.

Anticipated outcome: Systematic comparison of human prenatal and postnatal B lymphopoiesis, integrating gene expression and epigenetic profiles obtained on the same platforms, has not been performed before. This will identify whether prenatal B-progenitors display gene expression and chromatin accessibility patterns, which mimic leukaemic cells. These studies will also allow us to determine whether MLL-AF4 is sufficient to transform prenatal progenitors to give an aggressive ProB iALL and will identify genes/pathways that provide a permissive context for MLLr transformation. The lack of accurate iALL models that fully replicate human disease restricts rational drug development. This project will use human prenatal progenitors to create an iALL model for the first time to provide the right cellular context to recapitulate human iALL. This will allow downstream assays to understand the mechanisms of treatment resistance and the impact of prenatal specific genes on the biology of ALL. These results will be instrumental in helping the future development of targeted therapy for specific molecular pathways relevant for initiation, maintenance or propagation of human leukaemia.

Translational potential of the project: Although there has been much progress in treating ALL in children, there is still a subset of ALLs that are incurable. New therapeutic strategies are urgently needed for treatment-resistant childhood leukaemia, such as iALL. Current iALL treatments have acute and long-term treatment related morbidities, and have so far failed to improve survival in the past two decades[2,8]. This research project will provide fundamental insight into ALL pathogenesis and may pinpoint key vulnerabilities of treatment-resistant iALL, which can be exploited for targeted drug discovery and/or immunotherapeutic approaches. Although iALL is rare, we envisage that a similar strategy of placing scientific studies at the heart of identifying new treatment strategies could be used for other treatment-resistant childhood/adult leukaemias. Going forward, we will build a research programme focusing on childhood leukaemias where the need is greatest; of note, including strategies for treatment reduction as well as identification of new or repurposed drugs.

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30. Personalised Breast Cancer Screening - Development and Validation of a Novel Approach Using the High Dimensional Data Source QResearch and Linked Databases - Prof. Hippisley-Cox[1,2]

Primary Supervisor: Julia Hippisley-Cox

Additional Supervisors: Gary Collins, Mike Brady, Simon Lord, David Dodwell

Collaborators: Brian Nicholson, Simon Leedham, Claire Bloomfield

Eligibility: Track 1 or Track 2 students only are eligible to apply for this project.

Required Background: Trainee clinician or medical undergraduate.

Project Summary

Abstract

Despite breast screening (screening mammography) being offered in multiple countries, there is considerable debate regarding the harms and benefits of mammography and offering it universally to all women within specific age ranges[1]. Breast screening may lead to overdiagnosis (the detection of small tumours that would never cause harm but are nonetheless treated) with ensuing 'overtreatment' and false positives. There is also considerable variation in the interpretation of results from clinical trials and epidemiological studies in terms of the true effects of screening on breast cancer outcomes[2]. Risk-adapted breast screening is a relatively novel concept which considers that not all women have the same risk of developing or dying from breast cancer. It seeks to target those women that are most likely to gain from partaking in screening and minimise harms in those least likely to benefit by reducing screening in the latter[3]. However, such methods are controversial and there are challenges to their implementation[4]. Machine learning approaches have recently been shown to accurately predict breast cancer diagnoses [5,6], however, these are trained using imaging data and a small collection of clinical variables – furthermore, it is unclear how these could be integrated into a health system such as the NHS[4]. This DPhil project aims to pursue a 'big data' approach on multiple linked and datasets comprising primary care databases, individual women's genome sequencing data (parallel analysis), screening mammography imaging, and routine biopsy data. These linked datasets of UK-based women will be examined using a combination of classical epidemiological and machine learning methodologies to develop multi-factorial risk prediction models that could guide risk-adapted screening for breast cancer. A health economics analysis of any derived models would be performed to assess the costs and benefits from a healthcare system perspective.

Research objectives

1. Generate prediction models from general practice datasets (QResearch linked databases) that can stratify women in terms of their risk of developing invasive breast cancer within the next 5 years, and next 10 years
2. Comparative evaluation of 2 types of risk prediction model: 'traditional' epidemiological regression models and those from machine learning (ML)
3. Validation of derived models in external datasets (i.e. CPRD, a distinct primary care database)
4. Evaluation of derived models against extant risk prediction systems published elsewhere
5. As above, but risk of death from breast cancer in the same time frame
   a. Perform a parallel analysis of UK Biobank data to derive predictive models (statistical and ML) for breast cancer diagnosis and breast cancer death that integrate clinical, imaging and genetic sequencing data
   b. Through linkage of general practice records to hospital-held 'first screen' mammography images and biopsy images, derive machine learning algorithms (convolutional neural networks) that predict the risk of developing invasive breast cancer within the next 5-years, and the next 10 years
6. Compare power of predictive models based on integration of primary care data and imaging data with those based on primary care data alone
   a. Produce an 'optimised' risk prediction model for breast cancer in British women

i. Assess impacts on the NHS of implementing breast screening in accordance with the developed model(s), and compare these against age-based screening using a decision-analytic economic evaluation
ii. Estimate costs to NHS, possible effects on breast cancer incidence and breast cancer mortality

b. Perform qualitative analyses of doctors' and women's views on the derived screening models

The academic value of the research is embodied in the rigorous development and comparative evaluation of multiple risk prediction models that encompasses both 'classical' epidemiological approaches and the vogue (albeit incredibly powerful) methods of machine learning. The research will be completed under the supervision of world-leading experts in the fields of risk prediction models, big data, image analysis and breast cancer and is expected to lead to the publication of results that may have profound implications for screening policy. The award will foster unique and novel collaborations between primary care health scientists, data scientists/machine learning developers as well as GPs, oncologists and health economists with a strict emphasis on the multidisciplinary approach needed to undertake such health service-focused translational work.

Translational potential

To our knowledge, the proposed DPhil project represents the first attempt to unify multiple datasets from primary and secondary care institutions to derive and validate strategies for risk-adapted screening mammography, but also assess the economic impact of big data-driven risk models. It seeks to venture beyond what has previously been analysed in this field[5-8], i.e. a process encompassing database synthesis, model development, validation and economic analysis to provide a potential 'ready to deploy' model for risk-adapted breast screening, with the NHS in focus. It seeks to leverage the unique situation wherein Oxford hosts QResearch (the largest primary care database in the UK linked to cancer, mortality and hospital records and the source for widely implemented risk prediction models[9,10]) and an incredibly strong clinical DPhil programme with big data as a strategic vertical. Breast cancer is one of the commonest cancers diagnosed in UK women with >55,000 diagnoses per year and >11,000 deaths. Long-term survival has significantly improved in the past 30 years, which may be due to screening and or improved therapy. However, the attribution of impact from mammography versus improved treatment strategies is one of the most contentious issues in evidence-based medicine, with global debate. It is envisaged that the models derived from this work would represent rigorously developed and validated methods capable of altering screening practices in the United Kingdom and other countries, therewith offering the potential of improving the detection and outcomes of one of the world's leading causes of cancer death.

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31. Studying the roles of a chromatin remodelling factor (ATRX) in normal gene expression and in malignancy – Prof. Higgs[1,2,3]

Primary Supervisor: Douglas Higgs

Additional Supervisors: Richard Gibbons, David Clynes

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: A biomedical/biological background.

Project Summary

Abstract Thalassaemia is the most common form of inherited anaemia throughout the world. In all cases, it results from an imbalance in the production of the -like and -like globin chains of haemoglobin, leading to -thalassaemia and -thalassaemia respectively. The aim of our laboratory is to understand how the globin gene clusters are normally regulated during development and differentiation and how this is perturbed in patients with thalassaemia. By approaching these questions, we are also developing a general understanding of the principles by which mammalian genes are normally switched on and off and how these processes go awry in acquired and inherited human genetic diseases, including cancer.

During the course of this work, we have identified about 130 patients who have a rare form of -thalassaemia which occurs in the context of a pre-malignant condition called the myelodysplastic syndrome (MDS). Many of these patients go on to develop acute myeloid leukaemia. These patients have no pre-existing forms of -thalassaemia (AT) and so this condition is acquired specifically in the pre-malignant clones of cells in MDS: hence the condition is referred to as the ATMDS syndrome. When we analyse the bone marrow cells of patients with ATMDS we find a distinct constellation of mutations in epigenetic readers, writers and erasers which are also found in other patients with MDS but, importantly, in addition, most patients with ATMDS have mutations in a chromatin remodelling factor called *ATRX*. This protein was discovered in our laboratory in 1995 as a cause of X-linked -thalassaemia associated with developmental abnormalities (ATR-X syndrome) and *ATRX* has more recently been recognised as a tumour suppressor gene in a wide variety of malignant tumours including glioblastoma, melanoma, pancreatic neuroendocrine tumours and a wide range of sarcomas.

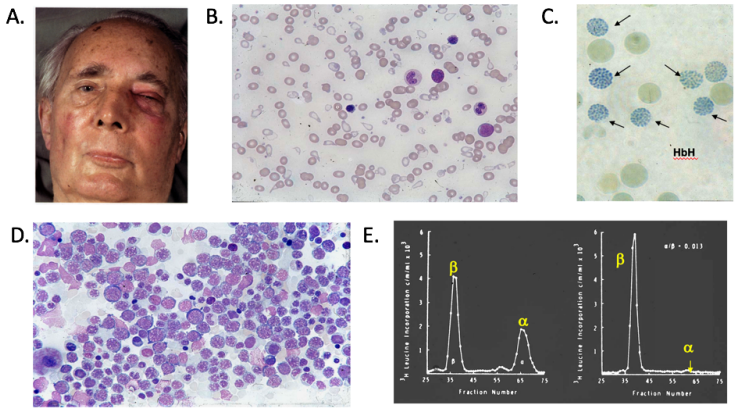

Figure 1. A.. A patient with MDS B. the blood film showing hypochromic microcytic red blood cells. C. Cells containing HbH inclusions which are tetramers of excess β-globin chains (β_4) seen in patients with α-thalassaemia. D. a bone marrow smear showing the features of MDS. E. Globin chain synthesis in a normal individual (left) and a patient with ATMDS (right). There is an almost complete absence of α-globin synthesis in this individual.

By understanding the role of ATRX *in vivo*, we hope to understand its normal role in gene expression and how this is perturbed in malignancy. Analysis of naturally occurring mutations often provide important clues to mechanism of disease. The key scientific question in this project is how do mutations in ATRX down regulate -globin gene expression in ATMDS syndrome and why is the effect so much greater in ATMDS compared with ATR-X syndrome. We have been studying both primary cells from patients with ATMDS or ATR-X syndrome and developing much needed erythroid cell models of these diseases. This has been challenging for a variety of reasons but recently we have shown that using single cell analysis we are now able to identify a sub-population of erythroid cells that appear to be more affected by ATRX mutations than others and we are currently

investigating why this should be so. Some clues to this will come from analysing the impact of other genes that are mutated in ATMDS syndrome: appropriate cell lines in which such genes have been mutated individually and in combination are now edited and available for further studies. The aims of this project will therefore be to further characterise primary cells and the recently established erythroid cell models of ATMDS syndrome using transcriptional, epigenetic and chromosome conformation studies to analyse how -globin expression is perturbed in this severe, acquired form of thalassaemia. All of such experimental approaches are well established in our laboratory. This project will contribute to our understanding of globin gene regulation, the general mechanism(s) by which chromatin remodelling factors normally work and how they may contribute to malignant diseases when mutated.

Research objectives and proposed outcomes The role of chromatin remodelling factors is at the forefront of research into gene regulation and cancer. We contribute to many aspects of gene regulation both transcriptional and epigenetic aspects via publication in high impact journals and engagement in international meetings. We have expertise on a wide range of approaches to transcriptional and epigenetic biology in our laboratories. We have well established collaborations with clinical haematologists in the UK, EU and US who have been contributing to this programme of work and we have an established network of collaborators in the transcriptional and epigenetic areas of research.

Translational potential of the project and relevance to cancer Since we originally identified the ATRX gene we have also shown that the protein is part of a protein complex together with a histone chaperone DAXX and the histone variant H3.3. Mutations in all three components of this complex have now been associated with a wide variety of malignant tumours. Almost all of such tumours maintain their telomeres via the so-called alternative (ALT) pathway of telomere maintenance. This aspect of ATRX is also being studied independently by a member of Oncology (Dr David Clynes) in collaboration with Professor Richard Gibbons (Co-supervisor). The current proposal aims to study the role of ATRX in gene expression. There seems little doubt that understanding the normal biological role of this complex will be of importance in understanding its role in the development of cancer.

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32. R-loops: New Therapeutic Targets in Cancer- Dr. Gromak[1,2,3]

Primary Supervisor: Natalia Gromak

Additional Supervisors: Tom Brown

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: A biomedical/biological background.

Project Summary

Abstract

Cancer is a disease associated with genome instability. Unusual RNA/DNA structures, R-loops, have recently emerged as important triggers of genome instability. These structures are formed during transcription and they are implicated in a number of important biological functions, including transcription, DNA replication and epigenetics. However, mis-regulation of R-loops can lead to human pathologies, including neurodegeneration and cancer. In particular, we showed that R-loops are up-regulated in cancer resulting in replication stress and genomic instability (Kotsantis et al., 2016). To investigate the function of R-loops in cancer, we have recently established high-throughput affinity purification followed by mass-spectrometry to define R-loop binding proteome in cancer cells (Cristini et al., 2018). Among these factors, we identified 33 DEAD/H helicases, all over-expressed in cancer. This project aims to investigate the molecular mechanisms underlying the role of R-loops and R-loop helicases in cancer using genome-wide, gene-specific and chemistry approaches. This project will allow us to apply our expertise in R-loop biology, chromatin dynamics and nucleic acid chemistry and to use the state-of-the-art technology to understand the molecular basis underlying cancer. This project will also help to uncover the therapeutic and prognostic potential of R-loops and R-loop helicases in cancer.

Introduction And Preliminary Data

Genome instability is one of the hallmarks of cancer. Recently unusual RNA/DNA structures, R-loops, have emerged as important triggers of genome instability. R-loops are formed during the process of transcription when nascent RNA hybridizes to the DNA template, forming an RNA/DNA hybrid, behind the elongating RNA polymerase. R-loops are found in all living organisms, and they play crucial biological functions, including transcription, replication, gene expression and generation of antibody diversity. However, their dysregulation results in DNA damage and genome instability which contribute to R-loop-associated human diseases, including cancer and neurodgeneration (Groh and Gromak, 2014). RAS genes are mutated in one third of human cancers, where they act as oncogenes. We recently showed that the most common HRAS mutation (G12) induces R-loop accumulation, causing replication stress and DNA damage (Kotsantis 2016) (Figure 1A). To get further insights in R-loop functions in cancer, we recently established high-throughput affinity purification followed by mass spectrometry (MS) to define R-loop binding proteome (Cristini et al., 2018). Using this approach we identified a new class of R-loop regulators, the DEAD/H family of helicases (Figure B-C). Interestingly, these DEAD/H helicases are over-expressed and amplified in different types of cancer (Figure 1D). Among them, we characterized the role of DHX9, which was previously implicated in cancer (Lee et al 2016), in preventing R-loop-associated DNA damage. Our findings suggest that R-loops and DEAD/H helicases may be the key regulators in cancer cells and represent potential therapeutic targets.

Research Objectives

The AIM of this research project is to investigate the molecular mechanism underlying the role of R-loops and RNA/DNA helicases in cancer. In particular we will address **the following research questions**:

AIM 1: Which R-loop helicases are implicated in cancer?

Outcome: Using CRISPR-Cas9 screens and genome-wide ChIP/DIP-seq approaches, we will establish which of the 33 DEAD/H helicases, up-regulated in cancer, are directly involved in R-loop-mediated genomic instability.

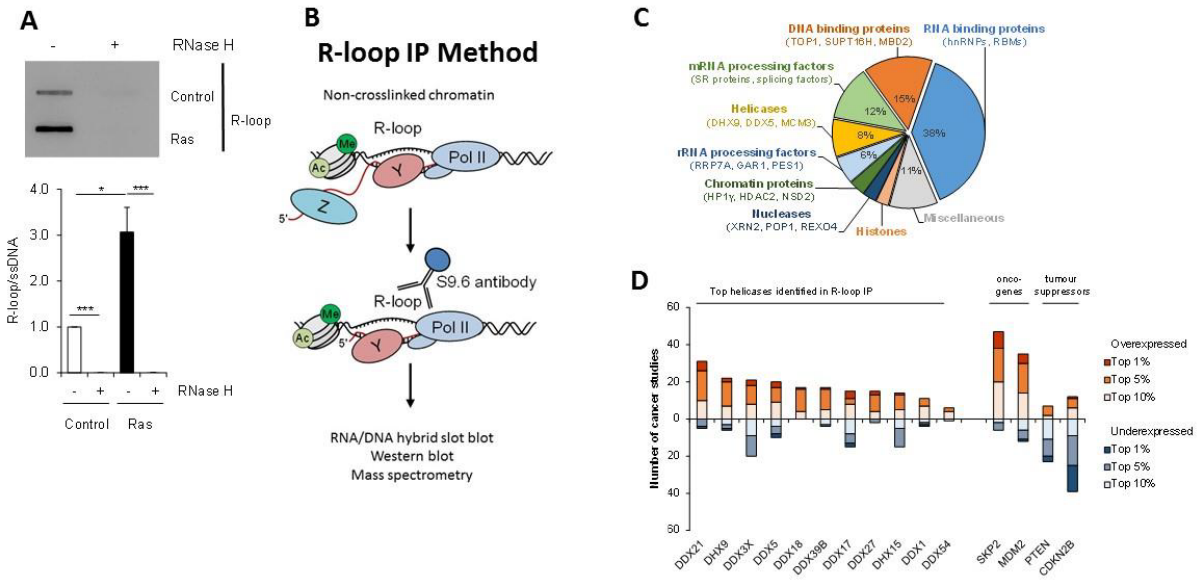
AIM 2: Which molecular complexes are involved in R-loop resolution in cancer?

Outcome: We will characterise the molecular complexes, containing DEAD/H helicases, involved in R-loop resolution (R-loop resolvasome) in cancer, using biochemical and proteomic approaches, followed by validation in vivo.

AIM 3: How R-loops can be specifically targeted in cancer?

Outcome: We will explore the possibility of therapeutic targeting/modulating the level of R-loops and the expression level of R-loop helicases in cancer. This will be carried out in collaboration with Prof. Tom Brown (Department of Chemistry, University of Oxford), expert in nucleic acid chemistry. In particular, we will develop chemical/small molecule strategies interfering with R-loop formation in vivo.

Deciphering the molecular link between RNA/DNA helicases and R-loops in cancer cells will be a new line of research in our lab. This project will allow us to apply our expertise in R-loop biology and chromatin dynamics and to use the state-of-the-art technology to understand the molecular basis underlying cancer. Employing the nucleic acid chemistry expertise of Prof. T.Brown (Chemistry Department), this project will also help to determine if the link between pathological R-loops and RNA/DNA helicases can be further utilised in early cancer diagnostics and therapeutics. Preliminary data presented in the proposal and our established expertise in all techniques proposed in this grant (genome-wide ChIP/DIP-seq, proteomics, biochemistry and RNA/DNA chemistry) provide the feasibility to this research proposal.



RNA/DNA helicases are enriched in the R-loop interactome and over-expressed in cancer
*(**A**) R-loops are induced in Ras-induced cancer model. Slot blot analysis of DNA from BJ-HRAS cells after Ras induction for 72 h, +/-RNase H (Kotsantis et al., 2016). (**B**) R-loop immunoprecipitation method. (**C**) Proteins identified in the R-loop IP by MS (Cristini et al., 2018). (**D**) Transcriptional overexpression of R-loop-associated helicases in cancer (data from ONCOMINE database).*

Translational Potential Of The Project

Fundamental role of R-loops in cancer biology represents a unique and novel concept in the cancer field. Therapeutic targeting of R-loops is an exciting and promising approach which is currently being exploited in the field of neurodegenerative diseases, with my lab being involved in this work. Therefore we anticipate that our expertise in R-loop field combined with nucleic acid chemistry expertise of Prof. T.Brown's group will put this project at the forefront of the cancer research.

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33. Dissecting immune responses to chemoimmunotherapy in non-small cell lung carcinoma – Prof. Van den Eynde[1,2,3]

Primary Supervisor: Benoit Van den Eynde

Additional Supervisors: Carol Leung and Mark Middleton

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: A biomedical/biological background.

Project Summary

Abstract
Our lab has developed new MAGE-targeting viral vectored cancer vaccines, with a chimpanzee adenovirus prime and a MVA boost, to induce strong CD8 CTL responses. Our pre-clinical data showed that the combination of the vaccines with chemotherapy and anti-PD1 checkpoint inhibitor stimulated robust anti-tumour specific immune responses and had high therapeutic efficacy in mouse tumour models. In partnership with CRUK, we will launch a clinical trial to study the therapeutic effect of the cancer vaccines against the MAGE-type antigens: MAGE-A3 and NY-ESO-1, in combination with standard of care chemotherapy and anti-PD1 checkpoint inhibitor in NSCLC. This project will analyse the immune responses and T cell receptor repertoire from NSCLC patients who received chemoimmunotherapy, including patients from different cohorts. The results will lead to a better understanding of the immune responses that associate with response or non-response to chemoimmunotherapy. This will have implications on future treatment of NSCLC.

Background
Non-small cell lung carcinoma (NSCLC) accounts for about 85% of all lung cancers and is the leading cause of cancer death. Overall survival of advanced NSCLC is less than 5% at 5 years. The standard of care in advanced NSCLC includes chemotherapy and anti-PD1 checkpoint inhibitor therapy. Anti-PD1 immunotherapy is now critical for treating NSCLC but only a subset of patients respond well to this therapy. The efficacy of checkpoint blockade depends on pre-existing anti-tumour CD8+ T cell responses. Cancer vaccines have the potential to induce tumour specific immune responses and can be combined with the standard of care therapies to reject tumours. Although a lot of effort have been made to generate an effective cancer vaccine over the last few decades, the results so far have been very disappointing, as most of these cancer vaccines failed to induce protective anti-tumour CD8+ cytotoxic T lymphocytes (CTL) in human. Here, we use a viral vectored vaccine platform, with a chimpanzee adenovirus prime and a MVA boost, which has been proven to induce effective CD8+ T cell responses against *Plasmodium falciparum* in humans (1). Extending the success of this vaccine platform to tumour antigens will provide new hopes in cancer vaccine development (2) (3).
A successful cancer vaccine needs to target tumour antigens that are expressed on tumours but not on normal tissues. Cancer-germline antigens are expressed in a wide variety of tumours but not on normal tissues except for germline cells that are incapable to present antigens to the immune system due to the lack of MHC I molecules. These antigens encoded by cancer-germline genes are known as MAGE-type antigens. NY-ESO1 and MAGE-A3 are prototypical MAGE-type antigens and their responses have been shown in a number of patients. Importantly, MAGE-A3 is expressed in 30-50% of NSCLC.
Our lab has generated adenovirus and MVA expressing MAGE-A3, NY-ESO1 and their murine counterpart P1A. The efficacy of these viral vectored vaccines was tested in different mouse tumour models. We have promising pre-clinical data to show that the combination of chemotherapy, anti-PD1 checkpoint inhibitor and the cancer vaccines stimulated a robust anti-tumour specific immune responses, significantly inhibited tumour growth and improved survival. A clinical trial will be launched to investigate the vaccine efficacy in NSCLC patients. The therapeutic effect of the cancer vaccines in combination with the standard of care chemotherapy and anti-PD1 will be tested. There will be 2 arms in the trial, the vaccination arm and non-vaccination arm, with all receiving the chemotherapy and anti-PD1 therapy.

Research objectives
1. To characterise the immune response to chemoimmunotherapy in NSCLC patients.

2. To measure the immune responses induced by the treatment between vaccine and non-vaccine cohorts.
3. To analyse the T cell receptor repertoire and study the T cell function and memory phenotype with RNA and exome sequencing.

Methods

This proposed research will not only focus on the MAGE cancer vaccine trial, but on NSCLC patients receiving the standard of care immunotherapies. The Oxford Oncology service treats around tens of NSCLC patients per year with chemoimmunotherapy. We expect to have 5-8 patients joining the trial and additional samples will be acquired from other sites. There is also a large sample bank in Southampton with whom we collaborate on our CRUK Network Accelerator Award. With the availability of clinical samples from these different sources, the student/clinical researcher will analyse biopsies and blood cells collected at different time points on treatments to monitor the change in immune cell subsets - principally by flow cytometry. In addition, RNA and exome sequencing will be employed to study the T cell receptor repertoire and to identify the molecular differences between responders and non-responders. The T cell responses specific to MAGE-A3, NYESO-1 and neoantigens will be measured by ELISpot and intracellular cytokine staining assay.

Translational potential of the project

This project is directly integrated with a clinical trial that will be launched in 2020. The proposed work undertaken by the student/clinical researcher will be a crucial part in interpreting patient's immune responses. The immunomonitoring analysis will contribute to better understanding of the immunity that associate with response or non-response to the combination therapies. The results will have impact on clinical practice for advanced NSCLC patients in the future.

Training and mentoring arrangement

The laboratory research will be supervised by Prof. Benoit Van den Eynde and Dr. Carol Leung. Though our lab is relatively new in Oxford, our current graduate students are performing very well, with one DPhil student who will complete within the next 3 months. The student/clinical researcher will be given training in immunology and molecular biology techniques including flow cytometry, ELISpot and ELISA assays, sequencing work-flow etc. The immunology and molecular biology assays are well established in our lab and the student/clinical researcher will get training in sequencing work-flow from experts within the Ludwig Institute. Moreover, the student/clinical researcher will get training in bioinformatics analysis from specialists in the Old Road campus. The clinical supervision will be provided by Prof. Mark Middleton who has an excellent track record in supervising MDs and clinical research students. Apart from the scientific aspect of the research project, the student/clinical researcher will benefit enormously from the career development programme at the Ludwig Institute.

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34. Defining The Antigenic Basis of Graft Versus Leukaemia in Acute Myeloid Leukaemia (AML) Following Allogeneic Stem Cell Transplantation (Allo-SCT) – Prof. Vyas[1,2,3]

Primary Supervisor: Prof Paresh Vyas

Additional Supervisors: Persephone Borrow, Andrew McMichael, Chakraverty, Nicola Ternette

Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: A biomedical/biological background.

Project Summary

Abstract of the project

a. Fundamental scientific premise The most common curative cellular immune therapy in blood cancers is allogeneic stem cell transplantation (allo-SCT) (reveiwed in[1]). AML is the most frequent indication for allo-SCT (~10,000 patients worldwide/year). Allo-immune **g**raft-**v**ersus-**l**eukaemia (GvL) responses, involving donor T-cell reactivity towards recipient antigens, represent the major mechanism underlying the curative effect of allo-SCT2. Although 60% of AML patients are cured by allo-SCT, the major cause of treatment failure is disease relapse[3]. However, the mechanisms that dictate success or failure of GvL, in individual patients, are poorly understood.

Given the low somatic mutational burden in AML, **our hypothesis** is that protein variants mismatched between patient and donor, most likely unidentified germline minor histocompatibility antigens, will trigger a GvL response from donor T-cells. **Our specific aims** are to:

1. Identify all somatic and germline mismatched variants between patient and donor.
2. Test which mismatched peptide variants trigger a T-cell response in vitro from T-cells from post allo-SCT peripheral blood (PB) T-cells.
3. Track antigen-specific T-cell responses to these mismatched variants in individual patients and see if they correlate with elimination of disease and immune attack against other tissues in the patient (**g**raft **v**ersus **h**ost **d**isease – GvHD)

These data will provide the first information about the antigenic basis of GvL.

b. Translational benefit For the first time in the field we will:

1. Develop approaches to track GvL antigen-specific responses post allo-SCT in individual patients. This will then allow us to describe in patients who relapse if a GvL response never occurs, if a GvL response is initiated and then is extinguished; and in patients who are cured, if we can always detect a GvL response.
2. Track GvL to allow us to identify which donor genotypes can generate a GvL response. In the longer term this may help the field to test if donors likely to generate a GvL response give a better outcome in allo-SCT.
3. Design and test interventions to sustain/prevent extinction of GvL responses by tracking GvL responses.
4. Provide a knowledge base to begin to develop allo-reactive chimeric antigen receptor T cells (CAR-T cells) for use potentially in any malignancy.

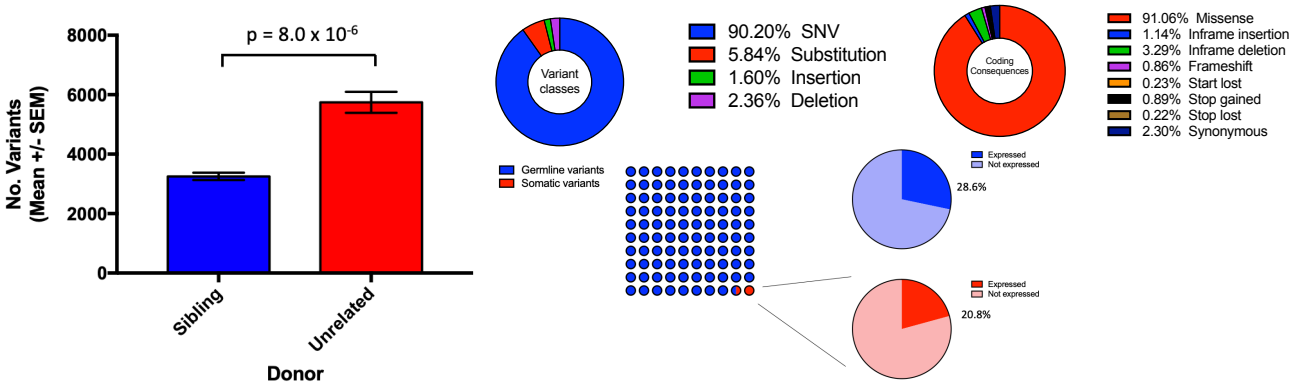
How will we identify the antigenic basis of GvL?

Samples: Over the last 12 years, we have been collecting bone marrow (BM) and PB samples from AML patients at diagnosis, pre-allo-SCT, and 3 monthly post allo-SCT, and donor cells and DNA pre-allo-SCT. We have identified 40 patients-donors with full sample sets. We are also prospectively biobanking diagnosis, pre-allo-SCT and post-allo-SCT BM and PB samples for two UK randomised allo-SCT trials (EudraCT numbers: 2017-004801-42 2018-001012-30; total patient numbers 652 in the UK; 158 patients already recruited and sequential BM and PB samples being biobanked) and will use these samples to validate our findings from the initial cohort.

Preliminary data: On 15 patient-donor pairs we performed exome sequencing on AML samples at diagnosis and donor DNA. We identified all somatic and germline variants mismatched between patient and donor (**Figure 1**). We performed RNA-sequencing on AML cells and identified mismatched variants expressed in AML cells. (**Figure**

1). We used Vyas Lab integrated germline and somatic variant caller pipelines integrated with RNA-Seq pipelines developed in collaboration with Drs Supat Thongjuea (WIMM) and David Wedge (BDI)

Figure 1: Left, mean number of mismatched variants (germline and somatic) in 15 patient-donor pairs. There are ~3200 mismatched variants per patient-sibling donor pair and ~5850 mismatched variants per patient-HLA-ClassI/II matched donor pair. On average mismatched variants were detected in 2132 genes per patient. Middle top, the classes of variants are depicited. SNV=single nucleotide variant. Right top, the predicted consequences in protein structure of variant. Below right, shows 98.7% of mismatched variants were germline. 28.6% of mismatched variants were detectable in RNAseq reads from AML cells.



We collaborated with Dr Ternette (funded by a grant from Human Immunology Discovery Initiative – HIDI) to identify peptides bound to HLA Class I and II on AML blasts by immunoprecipitation of HLA Class I and Class II, elution of peptides followed by mass spectrometry (MS). An average of 2132 genes contain variants/patient. 54.4% of genes were expressed in AML blasts but HLA bound peptide(s) were detected for only 2.8% of genes. Given this discrepancy we infer that the MS-detectable immunopeptidome is missing potential GvL antigens. Therefore, we performed *in silico* HLA Class I and II-binding prediction using NetMHCpan 4.0[4] and NetMHCIIpan 3.0[5], which utilise artificial neural networks trained on HLA-binding data. Putative epitopes were identified by integrating whole exome-seq and RNA-seq and patient specific HLA alleles. To assess the performance of *in silico* prediction, the binding affinity of peptide sequences identified from MS was assessed using the computational approach above. For Class I, 72% peptides that we identified by MS were predicted as high affinity binders computationally.

Research objectives and proposed outcomes.

Work going forward in the studentship: All T-cell immunology experiments are performed in collaboration with Profs Borrow/McMichael/Chakraverty/Dr Ternette. We will focus initially on 10 patient-donor pairs who are HLA-A02*0201 where the Class I peptide algorithms are most accurate.

(a) Detecting T-cell responses. We will test patient T cell recognition of mismatched peptide pools (10-50 peptides spanning sequences containing putative HLA-I and -II epitopes) from the 10 patient-donor pairs using ex vivo and cultured interferon □ ELISpot assays. Responses will be further characterised by intracellular cytokine staining and evaluation of CD107a expression (a marker of T cell granule exocytosis/activation) on CD4 and CD8 T cells following peptide stimulation of PB T cells at different points post allo-SCT (4 time points; 3, 6, 9 and 12 months post allo-SCT). We will also test T cell recognition of autologous AML cells with/without peptide pulsing. All these techniques are routinely used in the Borrow and McMichael laboratories.

(b) Tracking antigen-specific anti-AML multimer T-cell responses. We will make HLA-peptide multimers to peptides to track antigen-specific anti-AML multimer$^+$ T-cells pre-transplant and post allo-SCT and combine multimer staining with markers of differentiation (e.g. CD27, CD28, CD45RA/RO, CD57) and functional state (e.g. Ki67 - proliferation; PD-1, LAG3, TIM3 - exhaustion) comparing to non AML-reactive T-cells (e.g. CMV-specific T cells).

(c) Single cell T-cell receptor (TCR) and T-cell transcriptome and T-cell cell surface marker profiling. We will use 5' 10X Genomics single cell analysis to identify the TCR repertoire,T cell transcriptome and T cell surface markers (CITE-Seq) expressed by flow-sorted PB antigen-specific T-cells, which we will identify by *ex vivo* staining with peptide-HLA multimers, or on the basis of upregulation of activation markers (CD25/CD137 for CD8+ T cells and CD25/ICOS for CD4+ T cells) following peptide stimulation. A similar number of non-AML reactive memory CD4+ and CD8+ T cells will be analysed in parallel for comparison. We will also perform bulk TCR sequencing of total memory CD4+ and CD8+ T cell populations, pre- and post-allo-SCT, to give a more in-depth picture of changes occuring in the T cell repertoire after SCT. This approach will enable us to track expansion of the multimer$^+$ cells within the total repertoire and identify other clones that expand with similar kinetics. All these immunological

techaniques are routine the Borrow and McMichael labs and single cell techniques and computational analyses routine in the Vyas laboratory.

(d) Functional testing of antigen-specific anti-AML TCRs in donor PBLs. To prove that putative GvL antigens are recognised by presumed cognate TCRs, we will transduce T cell lines or donor PBLs with lentivectors encoding relevant TCRα and β chains and test for cytotoxicity and cytokine production following incubation with patient AML cells +/- pulsing with relevant/irrelevant peptides.

(e) Validation. Epitopes recognised in the initial 10 patient-donor pairs will be tested for recognition in other patients using samples from the ongoing clinical trials (EudraCT numbers: 2017-004801-42 2018-001012-30). The key question here is to understand if T cell responses are unqiue to each patient-donor pair or if there are shared antigenic determinants between patient-donor pairs.

What we will achieve: Put Oxford in an international leadership position in defining and tracking allo-immune GvL. It has the potential to transform allo-SCT, the most common curative form of immunotherapy in blood cancers. It also has the potential to inform design of HLA-matched CAR T cells that recognise allo-restricted antigens on any tumour cells.

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35. Identification of radiation-induced neoantigens in pre-clinical and clinical models – Prof. Higgins[1,2]

Primary Supervisor: Geoff Higgins

Additional Supervisors: Nicola Ternette, Tim Humphrey

Eligibility: Track 1 and Track 2 students only are eligible to apply for this project.

Required Background: A clinical trainee or medical undergraduate.

Project Summary

Abstract
The addition of immune checkpoint inhibitors to radiotherapy treatment has recently been shown to increase overall survival in lung cancer patients, and has now become the standard of care worldwide. The expression of antigens that are recognised by self-reactive T cells is essential for immune-mediated tumour rejection by immune checkpoint blockade therapy. This project seeks to identify whether radiation induces the expression of otherwise undetectable neoantigens. Pre-clinical and clinical components will characterise neoantigen presentation in cell lines and tissue samples at baseline, during and after radiotherapy. Identifying commonly occurring, radiation specific neoantigens might enable the targeting of specific therapies targeted against these neoantigens**.**

Research Proposal
It is increasingly recognised that radiation kills tumour cells not only by generating lethal DNA damage, but also by triggering immunogenic cell death. Pre-clinical evidence suggesting that radiotherapy (RT) may increase an anti-tumour immune response has been confirmed in a large phase III randomised controlled trial showing that the addition of Durvalumab (a PD-L1 immune checkpoint inhibitor) to chemo-radiotherapy led to a dramatic improvement in disease-free survival and overall survival in patients with stage III non-small cell lung cancer compared with placebo[1]. It is likely that other factors besides radiation induced up-regulation of PD-L1 contribute to this interaction.
We hypothesise that radiation leads to the presentation of previously absent immunopeptides, which could generate anti-tumour, radiation specific immune responses.
We propose testing the generation of immunopeptides prior to, during, and after radiation. The recently funded RadNet grant has led to the establishment of an immunopeptidomics facility. This group, led by Ternette have developed robust protocols to identify MHC bound peptides from cell lines and tissues[2].
Preliminary data from the Muschel and Ternette groups has suggested that radiation treatment increases the peptide repertoire in MC38 cells and that the sequences presented in irradiated cells are sourced from central pathways known to be affected by radiation (Figure 1).

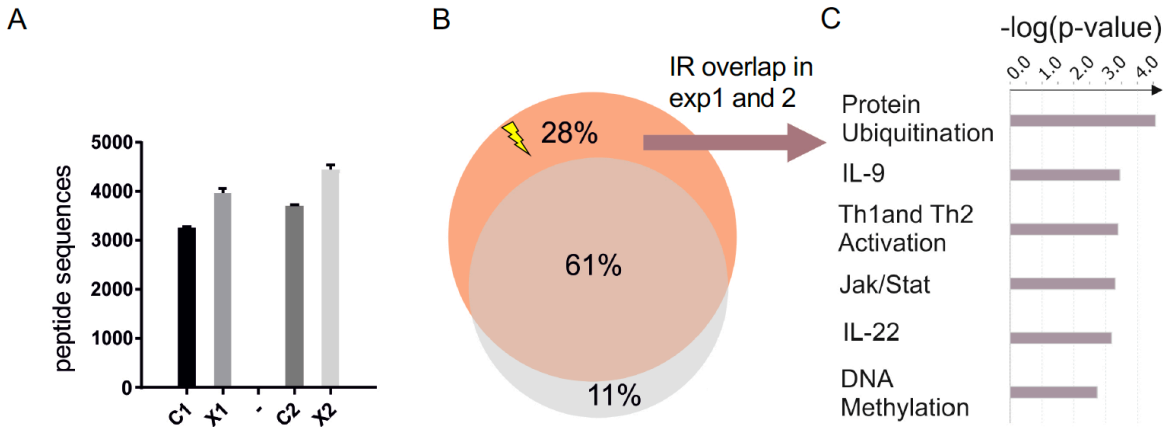

Figure 1. Radiation treatment and presentation of peptides sourced from proteins indicated in protein ubiquitination and DNA methylation. Mouse MC38 colon adenocarcinoma cells were exposed to radiation, or left untreated. Cells were harvested and MHC complexes purified using an antibody against mouse MHC class I (**A**) Between 3260 and 4511 peptide sequences were identified in two repeat experiments. Significantly more sequences were identified in the irradiated cells compared to the untreated control in both experiments. Experiment 1 and 2, X=irradiated; C=control). (**B**) Irradiated cells (orange and symbol) displayed 17% more unique MHC-associated peptides compared to control cells (grey). (**C**) The source proteins for the presented MHC-peptides identified in irradiated cells only are indicated in Protein Ubiquitination, Th1/2 Activation, Jak/Stat Signalling and DNA Methylation pathways according to Ingenuity pathway analysis (Qiagen).

The proposed project will involve several connecting elements.
1) Immunopeptidomic analysis will be undertaken in several established human tumour cell lines to establish whether any commonality exists in neoantigens present on irradiated cells. We will examine whether the addition of established and novel radiosensitising compounds alters the profile of presented neoantigens.
2) Irradiated murine models will be utilised to compare the 'actual' neoantigen repertoire identified by immunopeptidomic analysis, with the neoantigen landscape 'predicted' by RNA seq and TCR sequencing.
3) Blood and tumour samples will be obtained from patients undergoing radical radiotherapy at baseline, during, and after completion of radiotherapy. Head and neck, cervical, rectal and oesophageal tumours are routinely treated with definitive radiotherapy, and are particularly appropriate, since they are readily accessible for serial biopsying. We will assess whether there are overlapping features in the neoantigens detected a) between patient groups b) by cancer type, and c) with the pre-clinical findings.
The Clinical D.Phil student will have experience in tissue culture techniques and will have the option to learn *in vivo* studies. The Humphrey and Higgins groups have key group members with excellent track records in providing day to day student supervision (eg Sovan Sarkar and Remko Prevo). The immunopeptidomics core (Ternette) will provide full training to enable the student to process the acquired samples and give bioinformatics support to analyse the results. The Higgins group have experience in successfully supervising CRTFs undertaking clinical trials involving substantial translational components and sample analysis. We are well placed to provide comprehensive pre-clinical and clinical research training opportunities to the CRTF.

Translational potential
The successful identification of specific, commonly occurring radiation induced neoantigens could lead to the development of personalised treatments in combination with RT. These might include responsive vaccines, bispecific antibodies or CAR-T cell treatments.

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36. Defining a risk signature in peripheral blood T cells for the early detection of cancer – Prof. Barnes[1,2]

Primary Supervisor: Eleanor Barnes

Additional Supervisors: Paul Klenerman, Anette von Delft, Matthew Bottomley, Emannuele Marchi

Eligibility: Track 1 and Track 2 students only are eligible to apply for this project.

Required Background: A clinical trainee or medical undergraduate.

Project Abstract

Age and chronic infections are the two greatest risk factors for the development of cancer at a population level. It is generally assumed that this may be attributed to an increase in the burden of cancer driver genetic mutations over time with or without persistent inflammation. However, a decrease in effective immune surveillance and immune effector functions (particularly CD8+ T cells) that is known to characterise both old age (immunosenesence) and chronic infections (immune exhaustion) may also play a major contribution to cancer transformation and this is rarely evaluated. At the moment these two phenotypes are generally described as distinct entities. However, the overlap between immune senescence and T cell exhaustion is poorly understood [1]. Our aim is to investigate the interaction between immunosenesnece and T cell exhaustion and how they impact on cancer transformation and cancer risk.

Hypothesis: Age-related immune deficits in the T cell compartment contribute to increased cancer risk.
New tools are available to profile immune cell populations in unprecedented detail, particularly relating to characterisation of gene expression and the epigenome. In this proposal we will therefore:

(1) Establish an in vitro *models of CD8+ T cell exhaustion*[2] *and senescence-phenotypes*[3] by using standard markers of immune exhaustion (Flow cytometry), single cell RNA sequencing (scRNA-seq) and Assay for Transposon Accessible Chromatin (ATAC)/Chromatin immunoprecipitation Sequencing (ChipSeq) before and after differentiation to profile exhausted and senescent phenotypes. We will assess the validity and transferability of the model through comparisons of scRNA-seq and epigenetic profiles with those derived from:
a) CD8+ T cells in older people (>75 years),
b) Archetypal exhausted CD8+ T cell profiles associated with human chronic infection (HIV/HBV/HCV),
c) Archetypal senescent T cells associated with Cytomegalovirus (CMV) infection[4]. This will give a deep understanding of the overlap between immune exhaustion and immune senescence and define epigenetic and transcriptome signatures that may stratify cancer risks (evaluated in (2) below).

(2) *Assess the risk signature in two cohorts of patients at risk of cancer transformation:*
a) Skin cancer model: This is an established model at the Nuffield Department of Surgical Science , in which CD57+ T cells (a marker of immune senescence) has been associated with an increased risk of squamous cell carcinoma development in renal transplant patients[5],
b) Liver cancer model: A new cohort of patients with small hepatocellular cancer (nodules less than 3cm) will be recruited to establish the impact of T cell phenotypes on cancer development. This will provide critical real-world clinical data which will allow us to correlate deep immunological phenotypic changes with established clinical cancer risk[6].

(3) *Target changes associated with senescence and exhaustion within T cell subsets with novel epigenetic inhibitors (panel available from Structural Genetics Consortium (SGC)) to restore immune T cell function:* This 3rd aim is underpinned by an ongoing pilot project with UK Spine, aiming to establish simple assays that will be used

to identify key epigenetic regulators controlling epigenetic imprinting in T cell exhaustion and senescence[7]. A library of small molecule inhibitors selective for a range of epigenetic proteins will be used in established assays. For the first time, this would allow us to address the emerging problem of immune ageing and the risk of cancer - and potentially reverse it.

Translational Potential: The project is relevant in understanding the impact of immune surveillance on cancer risk at a molecular and cellular level. This applies directly in this case to skin and hepatic cancer – but clearly has wider implications. The project could provide a potential biomarker for cancer risk and for responses to immunotherapy, as well as potential new pathways and drug targets. Further, we are already exploring novel translational approaches within the project – we will learn from these not only if there are possibilities to impact on immune related cancer risk but also how human T cells respond to epigenetic modulations and define tractable key epigenetic regulators.

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37. Defining the role of structural maintenance of chromosome 5/6 complex in hepatitis B virus related hepatocellular carcinoma – Prof. McKeating[1,2,3]

Primary Supervisor: Jane A McKeating

Co-supervisors: Ester Hammond and Madhusudhan Srinivasan

Collaborators: Peter Ratcliffe, Xin Lu, Jo Parish and Ellie Barnes

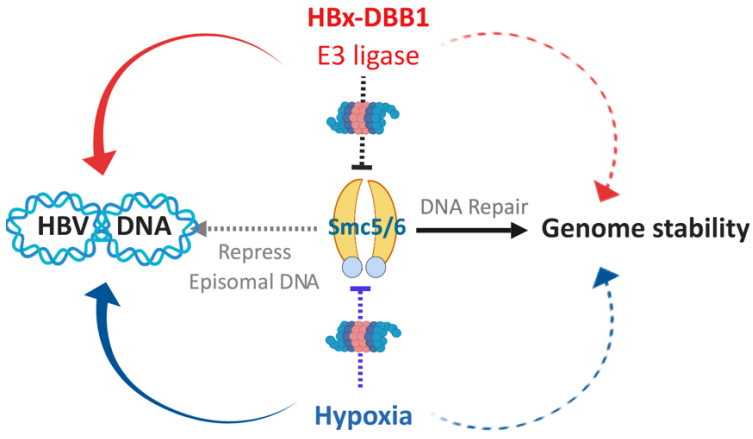
Eligibility: Track 1, Track 2, and Track 3 students are eligible to apply for this project.

Required Background: Either a biomedical/biological background.

Project Summary

Abstract
Hepatitis B virus (HBV) infection is a global health problem with >340 million infected individuals at risk of developing liver disease that can progress to hepatocellular carcinoma (HCC). However, the molecular pathways underlying viral oncogenesis are not well defined. Recent studies show that structural maintenance of chromosome 5/6 complex (Smc5/6) binds HBV episomal genomic DNA and inhibits transcription[1,2]. Along with condensin and cohesin, the Smc5/6 complex constitutes highly conserved molecular machines that play fundamental roles in chromosome organization, segregation and DNA repair[3] in all forms of life. However, the exact function of Smc5/6 is not well understood. Viruses frequently evolve pathways to evade host restriction factors and HBV encodes a regulatory protein X (HBx) that degrades Smc5/6 via DNA-damage binding protein 1 (DDB1) E3 ubiquitin ligase targeted proteasomal degradation and thereby promotes viral transcription.

The liver has a natural gradient of oxygen ranging from 9-3% in the peri-venous and peri-central areas, respectively. Cells adapt to low oxygen through a concerted transcriptional response regulated by hypoxia inducible factors (HIFs) that regulate a wide range of genes involved in energy metabolism. We recently discovered that a low oxygen (hypoxic) environment induces Smc5/6 degradation, providing a mechanism for HBV to infect hypoxic areas of the liver to evade this restriction factor. The discovery that HBx and hypoxia target Smc5/6 for degradation has important implications for understanding molecular mechanisms of hepatocellular carcinogenesis. Loss of Smc5/6 may predispose cells to genomic instability under conditions of DNA damage such as necroinflammation in chronic hepatitis B. We will investigate the role of HBx and hypoxic targeted Smc5/6 protein degradation in hepatoma DNA repair and genome stability.



Research objectives. HBV infection is associated with hepatocellular carcinoma, however, the underlying mechanisms are not fully understood. We hypothesise that HBx targeting of Smc5/6 for proteasomal degradation

contributes to the development of HCC and this is further exacerbated under hypoxic conditions. We will investigate this model in the following objectives.

1. To address the effect(s) of HBx or hypoxia mediated Smc5/6 degradation on genome stability and cellular response to DNA damaging agents. Studies in a range of organisms show that mutations in the Smc5/6 complex confer sensitivity to DNA damaging agents, however, our current understanding on the role of this complex in human cancer is limited. We have generated a tetracyline inducible-HBx liver progenitor HepaRG cell line that enables rapid and 'tunable' Smc5/6 degradation. We will use this model system to study the effect of hypoxia or HBx induced degradation of Smc5/6 on the cellular response to agents that induce double-strand breaks, methylated bases, topoisomerase-induced breaks and base damage. Defects in DNA repair pathways will be investigated by monitoring the rate of decline in γH2AX foci and we will assess genome instability by measuring the frequency of cells with micronuclei. Parallel studies will investigate Smc5/6 expression in HCC comparing tumour and adjacent non-tumour tissue.

2.To identify common or synergistic pathways of hypoxia and HBx targeted Smc5/6 degradation. We will investigate the role of HIFs in Smc5/6 proteasomal degradation using a panel of HIF modifying compounds. Recent studies reporting that inhibition of E3 ubiquitin ligase[1], NEDD8[4] and DDB1-HBx interaction with Nitazoxanide[5] rescues Smc5/6 from HBx-mediated proteasomal degradation provide compounds to investigate the mechanism underlying hypoxia targeted degradation of this complex.

3. Does Smc5/6 regulate other oncogenic DNA viruses? We will evaluate the role of Smc5/6 to restrict the replication of Epstein Barr virus and human papilloma virus in their respective target cells. We will use a panel of complementary approaches to investigate the role of Smc5/6 in viral restriction, including HBx over-expression and shRNA silencing technology. For studies in hypoxic conditions, we will engineer a hypoxia-inducible system to express Smc5/6 and determine the impact on viral transcription.

Anticipated results: To identify a role for Smc5/6 in HBV-associated HCC and to extend these observations to other oncogenic viruses.

Translational potential of project. The project builds on the diverse expertise of applicants in HBV (McKeating), hypoxia and DNA repair (Hammond), Smc complex and chromatin biology (Srinivasan) in collaboration with colleagues working on hypoxia (Ratcliffe), HCC (Barnes) and oncogenic viruses: Epstein Barr virus (Lu) and human papilloma virus (Parish). This project offers significant translational potential to assess the interplay between oncogenic virus replication, tumour hypoxia and role of Smc5/6.

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