Project 1-1

Project title: Discovery of potent agonist peptides for tumour-reactive T cells

Primary Supervisor: Ricardo A. Fernandes - ricardo.fernandes@ndm.ox.ac.uk
Additional Supervisors: Tao Dong – tao.dong@imm.ox.ac.uk

Project Summary

T cells probe the surrounding environment using the T-cell receptor (TCR) to scan peptides presented by the major histocompatibility complex. The nature and potency of the T cell response towards pathogens or tumour cells are determined by the signalling output from two distinct classes of immune receptors: the TCR and co-receptors, which includes activating and inhibitory checkpoint receptors such as CD28 or PD-1 and CTLA-4, respectively. The latest advances in single-cell sequencing have facilitated the identification of TCRs from clonally expanded, tumor-infiltrating T cells. However, the identification of agonist peptides is still notoriously challenging. This project aims to establish a framework to identify potent agonist peptides recognised by effector and regulatory T cells of interest, with a strong focus on identifying peptides recognised by TCRs from expanded tumour-infiltrating lymphocytes (TILs).

Project Overview

Background: Identifying antigens recognised by the TCR is challenging given the extreme diversity of the three individual components involved: peptide antigens, TCR and MHC. We aim to identify peptides, neoantigens and mimotopes, recognised by the TCR of clonally expanded CD8+ effector T cells in tumour settings (Fig. 1). To this end, we will engineer large (> 10^9) peptide-MHC libraries to be displayed at the surface of yeast cells, after which we will use an affinity-based screen to identify peptides recognised by TCRs of interest. This affinity-based approach will be complemented by a functional screen using an engineered system in mammalian cells. In this recently developed approach, the peptide-MHC library is fused to a CAR-like signalling module and displayed in T cells. This functional-based selection hijacks the unique sensitivity and specificity of the CD28/CD3 signalling modules to report on a productive TCR/pMHC interaction. Sorting of cells based on the upregulation of activation markers such as CD69 and CD25 will be used to isolate agonist peptides of different potency. The combination of affinity- and activity-based selections will guide the identification of potent agonist mimotopes, self-peptides or neoantigens using custom-built algorithms to rank closely related wild-type peptides. The identification of peptides recognised by tumour-reactive T cells will facilitate their expansion and detection using peptide-MHC molecules. Moreover, following isolation or activation with agonist peptides, tumour-reactive T cells will be characterised using single-cell transcriptomics and proteomics, for

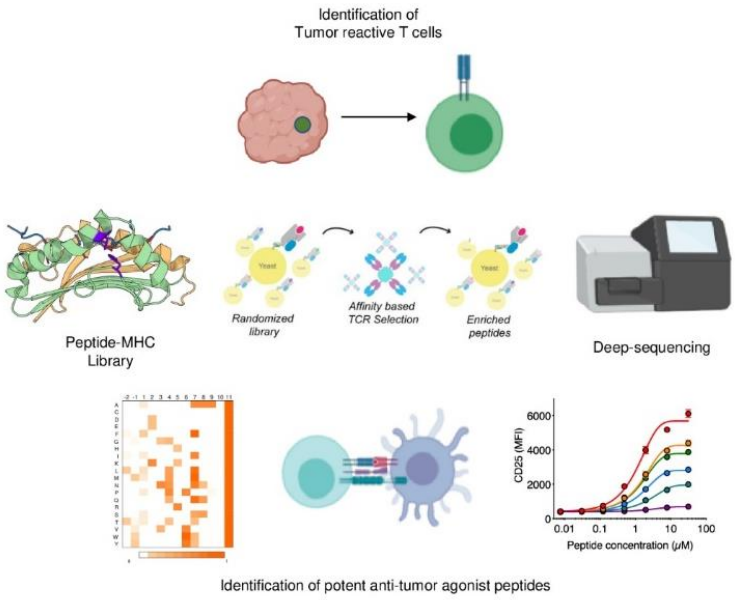


Figure 1. General overview of the experimental approach to discover peptide antigens to elicit robust anti-tumor T cell responses.

example. Agonist peptide identification combined with single-cell sequencing and quantitative proteomic analysis of relevant T cells will expand our current understanding of the role of diverse T cell subsets during an anti-tumour immune response. Furthermore, the discovery of disease-related agonist peptides opens the possibility to modulate T cell responses by peptide immunisation, an essential first step towards achieving in vivo expansion and activation of tumour-specific T cells. This research plan thus aims to contribute towards the development of relevant immunotherapies in cancer settings and a better understanding of T cell function.

Research objectives

This research plan aims to develop a framework for identifying peptides recognised by T cells of interest with a particular focus on tumour-reactive T cells. Candidate peptide antigens will be extensively characterised *in vitro* with functional and biophysical assays. We anticipate two primary outcomes. First, we expect to identify potent peptide agonists which will facilitate the identification, isolation and activation of tumour-reactive T cells. Second, the engineered peptide-MHC library displayed by yeast and mammalian cells will be made readily available to the scientific community.

Translational potential

The discovery of agonist peptides is notoriously challenging and has limited the possibility of expanding tumour-reactive T cells *in vivo*. We expect that the described approach will establish a rapid and facile method to discover peptide antigens for tumour-reactive T cells. Checkpoint inhibition blockade using antibodies against PD-1 and CTLA-4 to enhance T cell activity has shown great promise in the clinic, but in most patients, this approach fails to produce durable responses. We anticipate the next stage of immunotherapy development to involve a combination of checkpoint blockade - eliciting broad but unspecific potentiation of T cell responses - with antigen-specific stimulation of tumour-reactive T cells. The identification of peptide antigens for T cells involved in anti-tumour responses is expected to guide the selection of TCRs for adoptive cell transfer and the development of high-affinity TCRs and peptide vaccines for immunotherapy.

Training opportunities

The student will receive training in molecular biology, protein design, expression, purification and biophysical characterisation and various cellular assays. Moreover, the student will be trained in protein engineering, library design and selection using yeast- and mammalian-display. T cell signalling assays will be used to validate candidate antigens, which will provide an opportunity for training in flow cytometry and RNA-seq. This training will allow the candidate to drive fundamental and applied research in academia and industry. At the end of this project, the candidate will be in a great position to lead the development of new protein drugs from conceptual design to implementation and thorough validation in an area of great interest in T cell biology and immunotherapy. The student will have full access to the facilities and resources available within the Department and across the broader community at the University of Oxford.

Supervisor

Dr Ricardo Fernandes studied Biochemistry at University of Porto, Portugal. Following his interest in understanding the molecular basis of immune receptor signaling, Dr Fernandes moved to the Laboratory of Professor Simon Davis at the University of Oxford to pursue a DPhil focused in exploring the first events that lead to T cell receptor triggering, a fundamental step in T cell activation. In 2012 Dr Fernandes was awarded the Graduate Research Prize by the Nuffield

Department of Medicine for his DPhil Thesis. In 2015, and after being awarded a Sir Henry Wellcome Postdoctoral Fellowship by the Wellcome Trust, Dr Fernandes moved to Stanford University, US, to work in the lab of Prof. K. C. Garcia. Now at Oxford, Dr Fernandes has focused in using structural and mechanistic information to explore signaling initiated by the TCR and immune checkpoint receptors.

Key Publications

1. Gee MH, Han A, Lofgren SM, Beausang JF, Mendoza JL, Birnbaum ME, Bethune MT, Fisher S, Yang X, Bingham DB, Sibener LV, Fernandes RA, Velasco A, Baltimore, D, Schumacher TN, Khatri P, Quake SR, Davis MM, Garcia KC. Antigen identification for orphan T cell receptors expressed on tumor-infiltrating lymphocytes. (2018) Cell. Jan 25;172(3):549-563.e16.

2. Sibener LV, Fernandes RA, Kolawole EM, Carbone CB, Liu F, McAffee D, Yang D, Su DF, Yu D, Dong S, Gee MG, Jude KM, Birnbaum ME, Goddard WA, Davis MM, Groves JT, Heath JR, Evavold BD, Vale RD, Garcia KC. Isolation of a structural trigger required for TCR signaling from analysis of non-stimulatory peptide-MHC ligands. (2018) Cell. Jul; 174 (3), 672-687. e27.

3. Saligrama N, Zhao F, Sikora MJ, Serratelli W, Fernandes RA, Louis DM, Yao W, Chien YH, Garcia KC, Davis MM. Opposing T Cell Responses in Experimental Autoimmune Encephalomyelitis. (2019) Nature. Aug; 572(7770):481-487.

4. Fernandes RA*, Li C*, Wang G, Yang X, Savvides CS, Glassman CR, Dong S, Luxemberg E, Sibener LV, Birnbaum ME, Benoist C, Mathis D, Garcia KC. Discovery of surrogate agonists for visceral fat Treg cells that modulate metabolic indices in vivo. (2020) eLife. Aug; 9:e58463

Project 1-2

Project Title: Enhancing anti-tumor T cell function by controlled inhibition of checkpoint receptor signaling

Primary Supervisor: Ricardo A. Fernandes - ricardo.fernandes@ndm.ox.ac.uk

Project Overview

In the past decade, immune checkpoint blockade has emerged as a major therapeutic advance in immunotherapy. However, only a small subset of cancer patients respond to checkpoint blockade, suggesting that a fundamental understanding of the basic mechanisms of immune checkpoint receptor signalling is elusive and that novel therapeutic drugs must be developed. Here, we aim develop a novel approach to potentiate T cell function and to mechanistically understand how checkpoint receptors dampen T cell function.

Regulation of T cell signalling by immune checkpoints such as PD-1 and CTLA-4 has been at the centre of recent breakthroughs in cancer immunotherapy. Signalling by PD-1 and CTLA-4 reduces T cell activity and contributes to an "exhausted" phenotype, severely compromising antitumor responses. In the case of PD-1, binding to PD-L1/2 triggers the tyrosine phosphorylation of signalling motifs and results in the recruitment of cytosolic phosphatases such as SHP1/2, which in turn reduce TCR and CD28 signalling. Strikingly, signalling by several immune receptors relies on the Tyr phosphorylation of ITAM/ITIM/ITSM signalling motifs. We hypothesize that tonic receptor phosphorylation and sustained signalling by 'ligand-experienced' receptors impacts T cell function and fails to be controlled by extracellular antagonist antibodies. To address this issue, we engineered a bi-specific molecule to recruit CD45, an abundant and promiscuous receptor tyrosine phosphatase, within close proximity of PD-1 (Fig. 1). In this approach, the phosphatase domain of CD45 acts intracellularly, in *cis,* on the p-Tyr residues of the PD-1 ITIM/ITSM motif, thus inhibiting sustained signalling. We have shown that *Receptor Inhibition by Phosphatase Recruitment* (RIPR), potentiates T cell activity beyond that seen with PD-1/PD-L1 antagonist antibodies, both in the presence and absence of PD-1 ligand-binding *in vitro*, and to reduce tumour growth in mouse models of small cell lung cancer and colon adenocarcinoma (Fernandes *et al.*, *Nature*, 2020). Here, we propose the development of the RIPR concept to dissect the role of PD-1 in T cell "exhaustion" and, in parallel, to expand this novel approach to target other key immune and cancer-specific receptors aimed at generating novel antitumor, RIPR-based, molecules.

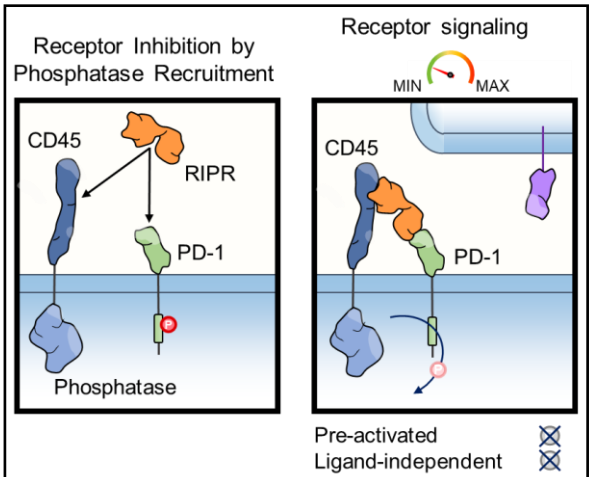


Fig. 1 | Receptor Inhibition by Phosphatase Recruitment. RIPR combines checkpoint blockade with CD45 recruitment. CD45 dephosphorylates receptors such as PD-1.

Aim 1. Determining a high-resolution, longitudinal, transcriptome of tumour infiltrating lymphocytes (TILs) upon RIPR-PD-1 treatment

Despite recent advances, the mechanistic basis of PD-1 inhibition remains incomplete. Reversal of the T cell exhaustion phenotype by antibody blockade appears to be inefficient. *Is T cell exhaustion irreversible?* RIPR-PD1 offers a new avenue to investigate the role of PD-1 and offers several

advantages over genetic deletion and antibody blockade, such as p-Tyr-specificity, controlled delivery and tunability. For this aim, we propose to sequence TILs, at different stages of an antitumor response and contrast this to the transcription profile upon RIPR-PD1 treatment. A longitudinal map will be created focusing on information obtained from single-cell transcriptome and TCR sequencing. The unbiased understanding of T cell dynamics before and after exposure to RIPR-PD1 will elucidate which pathways are turned "on" or "off" by PD-1 signalling. This information will help to determine a roadmap to investigate the mechanistic basis of immune checkpoint function with unparalleled granularity, and in the future will enable the development of novel therapeutic modalities.

Aim 2. Development of RIPR-based molecules to inhibit immune receptors

Can the RIPR concept be applied to other inhibitory receptors? We propose the development of a platform to systematically probe the RIPR effect in key immune inhibitory receptors. We will develop RIPR molecules to target other receptors of interest. Following *in vitro* testing of RIPR activity, promising candidates will be tested for antitumor activity *in vivo* in mouse models. Determinants of RIPR activity, such as epitope binding, receptor geometry and binding affinity, will be identified to better define the optimal RIPR architecture.

Impact

This proposal will define a roadmap for the PD-1 mediated control of T cell exhaustion and deliver novel molecules to impact antitumor responses with a strong potential for therapeutic application.

Training opportunities

The student will be mentored by Dr Fernandes and will receive formal training in protein engineering and protein expression and purification, flow cytometry, cell culture, wide range of assays to evaluate T cell function, T cell receptor repertoire analysis, RNASeq and bioinformatics.

Supervisor

Dr Ricardo Fernandes studied Biochemistry at University of Porto, Portugal. Following his interest in understanding the molecular basis of immune receptor signaling, Dr Fernandes moved to the Laboratory of Professor Simon Davis at the University of Oxford to pursue a DPhil focused in exploring the first events that lead to T cell receptor triggering, a fundamental step in T cell activation. In 2012 Dr Fernandes was awarded the Graduate Research Prize by the Nuffield Department of Medicine for his DPhil Thesis. In 2015, and after being awarded a Sir Henry Wellcome Postdoctoral Fellowship by the Wellcome Trust, Dr Fernandes moved to Stanford University, US, to work in the lab of Prof. K. C. Garcia. Now at Oxford, Dr Fernandes has focused in using structural and mechanistic information to explore signaling initiated by the TCR and immune checkpoint receptors.

Key publications

1. Fernandes RA, Su L, Nishiga Y, Ren J, Bhuiyan AM, Ali LR, Majzner R, Ohtsuki S, Rietberg SP, Yang X, Picton L, Savvides CS, Mackall, CL, Sage J, Dougan M, Garcia KC. Immune receptor inhibition through enforced phosphatase recruitment. (2020) Nature, Oct;586(7831):779-784

2. Fernandes RA*, Ganzinger KA*, Tzou J, Jonsson P, Lee SF, Palayret M, Santos AM, Chang VT, Macleod C, Lagerholm BC, Lindsay AE, Dushek O, Tilevik A, Davis SD, Klenerman D. A cell-topography based mechanism for ligand discrimination by the T-cell receptor. (2019) Proc Natl Acad Sci U S A. Jul; 116(28), 14002-14010

3. Chang VT*, Fernandes RA*, Ganzinger KA*, Lee SF*, Siebold C, McColl J, Jönsson P, Palayret M, Harlos K, Coles CH, Jones EY, Lui Y, Huang E, Gilbert RJ, Klenerman D, Aricescu AR, Davis SJ. Initiation of T cell signaling by CD45 segregation at 'close contacts'. (2016) Nat Immunol. May;17(5):574-82

4. Fernandes RA*, Yu C*, Carmo AM, Evans EJ, van der Merwe PA, Davis SJ (2010) What controls T cell receptor phosphorylation? Cell. 142: 668-669

Project 2

Project Title: Revolution in the Cellular Pathology of Kidney Disease

Primary Supervisor: Richard Cornall - richard.cornall@ndm.ox.ac.uk
Additional Supervisors: Katherine Bull - bullk@well.ox.ac.uk

Project Overview
The development of new therapies for kidney disease is limited by poor understanding of the pathology of disease at a cellular level. This contrasts with the emerging situation in cancer biology where knowledge of cellular biology, including the immune response, is starting to transform treatment. To make similar advances for patients with renal disease requires us to develop the same approaches to interrogate kidney tissue cellular pathology. This will require (i) the study of samples from sufficient numbers of patients to control for genetic and environmental heterogeneity; and (ii) the development of a variety of new approaches to pathology, including spatial molecular imaging.

Over 200 kidney transplants are performed in the Oxford Transplant Unit each year. Over 40% of the patients waiting for a transplant have a primary kidney disease with a known risk of disease recurrence in the donor kidney post transplantation. For example IgA nephropathy and some forms of focal segmental glomerulosclerosis (FSGS). FSGS recurs in 25% of cases, sometimes within hours or days post transplantation, while IgA recurs histologically in over 50% of patients by 4 years post-transplant. Access to paired pre- and post-implantation transplant kidney biopsy samples presents a unique opportunity to study the very earliest pre-clinical stages of active renal disease in previously normal kidneys and develop new approaches that can then be applied more widely in renal disease.

On each paired sample, we will perform:

(i) Renal cortex spatial scRNA-seq comparing pre-implantation healthy donor tissue to samples post-transplantation with alignment to conventional morphology (Prof Jens Rittscher). This will involve a collaboration in spatial imaging with Beijing Genomic Institute.
(ii) Cellular podocyte and glomerular proteomics using laser capture micro-dissection and mass spectrometry of over 4000 proteins (with Dr Roman Fischer, TDI).
(iii) Validation. Combined analysis of these datasets will inform the design of disease specific high throughput multiplex imaging 40+ marker panels for tissue imaging

Disease associated signatures will be further validated by studying additional renal samples including existing biobank tissue, and used to identify potential drug targets and early biomarkers of recurrence or treatment response.

The Cornall and Bull labs have experience with a range of *in vivo* and *in vitro* models of immune and renal disease, in which credible candidate genes from the human samples may be explored mechanistically.

Training Opportunities
Renal medicine, Immunology and molecular and cell biology, Single cell transcriptomics, Proteomics, Spatial molecular imaging, with a collaboration with BGI, Coding and Statistical analysis.

Supervisors
Richard Cornall FRCP FMedSci is the Nuffield Professor of Clinical Medicine, Head of the Nuffield Department of Medicine, an MRC Investigator and Fellow of Magdalen College, Oxford. He is an Honorary Consultant Renal Physician. Richard's research interests include systemic autoimmune disease and the study tolerance, using transgenic and gene targeted models, genetic screens, B cell development and immunodeficiency, and the modulation of immune disease with checkpoint agonists. He is a co-founder of MIROBIo.

Katherine Bull FRCP is a Fellow at Exeter College, Oxford, an Honorary Consultant Renal Physician and an MRC Clinician Scientist in the Nuffield Department of Medicine. Katherine's research interests include the study of autoimmune and genetic renal disease, CRISPR gene editing, *in vivo and in vitro* models, and genomic approaches to the study of diseased tissue, including imaging and computational bioinformatic analysis.

Key Publications
1. Chen, A. *et al.* Large field of view-spatially resolved transcriptomics at nanoscale resolution. *Biorxiv* 2021.01.17.427004 (2021) doi:10.1101/2021.01.17.427004.

2. Davis, S., Scott, C., Ansorge, O. & Fischer, R. Development of a Sensitive, Scalable Method for Spatial, Cell-Type-Resolved Proteomics of the Human Brain. *Journal of proteome research* 18, 1787–1795 (2019).

3. Moroni, G., Belingheri, M., Frontini, G., Tamborini, F. & Messa, P. Immunoglobulin A Nephropathy. Recurrence After Renal Transplantation. *Front Immunol* 10, 1332 (2019).

4. Ferreira, R. M. *et al.* Integration of spatial and single cell transcriptomics localizes epithelial-immune cross-talk in kidney injury. *Jci Insight* 6, (2021).

Project 3

Project Title: Hypoxia inducible factors: a new therapeutic target for the treatment of respiratory viruses

Primary Supervisor: Jane McKeating - jane.mckeating@ndm.ox.ac.uk
Additional Supervisors: Teresa Lambe - teresa.lambe@paediatrics.ox.ac.uk; Christopher Schofield - christopher.schofield@chem.ox.ac.uk

Project Overview
Respiratory infections in humans are responsible for a significant proportion of global deaths, approximately 4.25 million/year, mostly in children and older adults. Viruses are responsible for the majority of these infections and new broad-spectrum active anti-viral agents are urgently needed to prevent infection associated morbidity and mortality. The COVID-19 pandemic, caused by the novel coronavirus SARS-CoV-2, has highlighted the importance of understanding fundamental host processes that viruses exploit to infect the respiratory tract. Virus replication is shaped by the cellular microenvironment and one important factor to consider is local oxygen tension, where the hypoxia inducible transcription factors (HIFs) regulate transcriptional responses to low oxygen or hypoxia. Viruses are dependent on their host cell transcription and translational machinery to replicate and understanding how HIFs impact the viral life cycle can facilitate the design and evaluation of new therapies.

We have demonstrated that the HIF-a prolyl hydroxylase inhibitor (PHI) Roxadustat, that stabilizes HIFs, potently inhibits SARS-CoV-2 entry and replication in lung epithelial cells. Recently we have demonstrated in vivo that Roxadustat treatment reduces the burden of infectious SARS-CoV-2 in the upper and lower respiratory tract and associated pathological damage in the Syrian golden hamster model. In this project we will extend these studies to include other respiratory pathogens including influenza, rhinovirus and respiratory syncytial virus – viruses which cause a significant health burden globally. Elucidating the conserved mechanisms underlying the transcriptional regulation of different virus families will help define the best therapeutic options.
This project will be co-supervised by Jane McKeating, Teresa Lambe and Chris Schofield who provide complementary expertise in molecular virology, hypoxia biology, respiratory immunology and chemical biology/medicinal chemistry. The interdisciplinary project will provide a unique training environment to establish pre-clinical models to evaluate the impact of PHI treatment on the replication of a range of respiratory viruses. A range of techniques will be offered and depending on the research interests of the student, they will be trained in virus replication models, preclinical mouse models, immunogenicity assessment including cellular immune assays and humoral immune assays and biological aspects of medicinal chemistry. Transferable skills including oral presentations at joint lab meetings, critical reviewing of published scientific literature by contributing to journal clubs and scientific writing by reviewing and drafting manuscripts for publication.

Supervisors
Jane McKeating is Professor of Molecular Virology at University of Oxford and her research studies the impact of hypoxia and circadian host signalling pathways on virus replication to uncover new therapies. Her laboratory has worked on clinical important viral pathogens including HIV, hepatitis B and C viruses and more recently SARS-CoV-2, with >200 papers (>31,673 citations, h-index 89).

Jane holds a visiting Professorship at the Technical University of Munich (2015-present) and is a founding fellow of Reuben College.

Teresa Lambe OBE is Head of Vaccine Immunology at Oxford Vaccine Group and directs a research programme to improve human health by understanding infectious diseases and bringing them under control through vaccination – stopping epidemics before they become pandemics. Prof. Lambe works on vaccines against globally important diseases, including Ebola, Influenza, MERS, and Crimean-Congo haemorrhagic fever (96 publications, h-index 45) and is one of the Principal Investigators overseeing the development of COVID-19 Oxford/AstraZeneca vaccine programme and was appointed as an honorary OBE for her services to Sciences and Public Health in Queen's Birthday Honours (2021) in recognition of this work.

Christopher Schofield is the Director of Chemistry at the Ineos Oxford Institute for Antimicrobial Research. His research has contributed to a chemical understanding of biological systems (>300 publications, >30,000 citations, h-index >80). His work has contributed to mechanistic and structural knowledge of enzymes of bio-medical interest and has enabled the development of new drugs, including antimicrobials and HIF stabilizing drugs for the treatment of anaemia. He has championed new training initiatives including the Synthesis for Biology and Medicine Doctoral Training Centre at Oxford, which links multiple pharmaceutical and agrochemical companies with basic chemical research in a collaborative and open manner as promoted by its patent free approach.

Key publications:

1. Wing, Keeley *et al* 2021. Hypoxic and pharmacological activation of HIF inhibits SARS-CoV-2 infection of lung epithelial cells. Cell Rep https://pubmed.ncbi.nlm.nih.gov/33852916/

2. Zhuang *et al.* 2020. Hypoxic microenvironment shapes HIV-1 replication and latency. Comms Biology https://pubmed.ncbi.nlm.nih.gov/32665623/

3. Liu *et al* 2020. Oxygen sensing and viral replication: implications for tropism and pathogenesis. Viruses https://pubmed.ncbi.nlm.nih.gov/33113858/

4. Voysey *et al* 2021. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. Lancet https://pubmed.ncbi.nlm.nih.gov/33306989/

5. Yeh et al. 2017. Molecular and cellular mechanisms of HIF prolyl hydroxylase inhibitors in clinical trials. Chem Sci https://pubmed.ncbi.nlm.nih.gov/29435217/

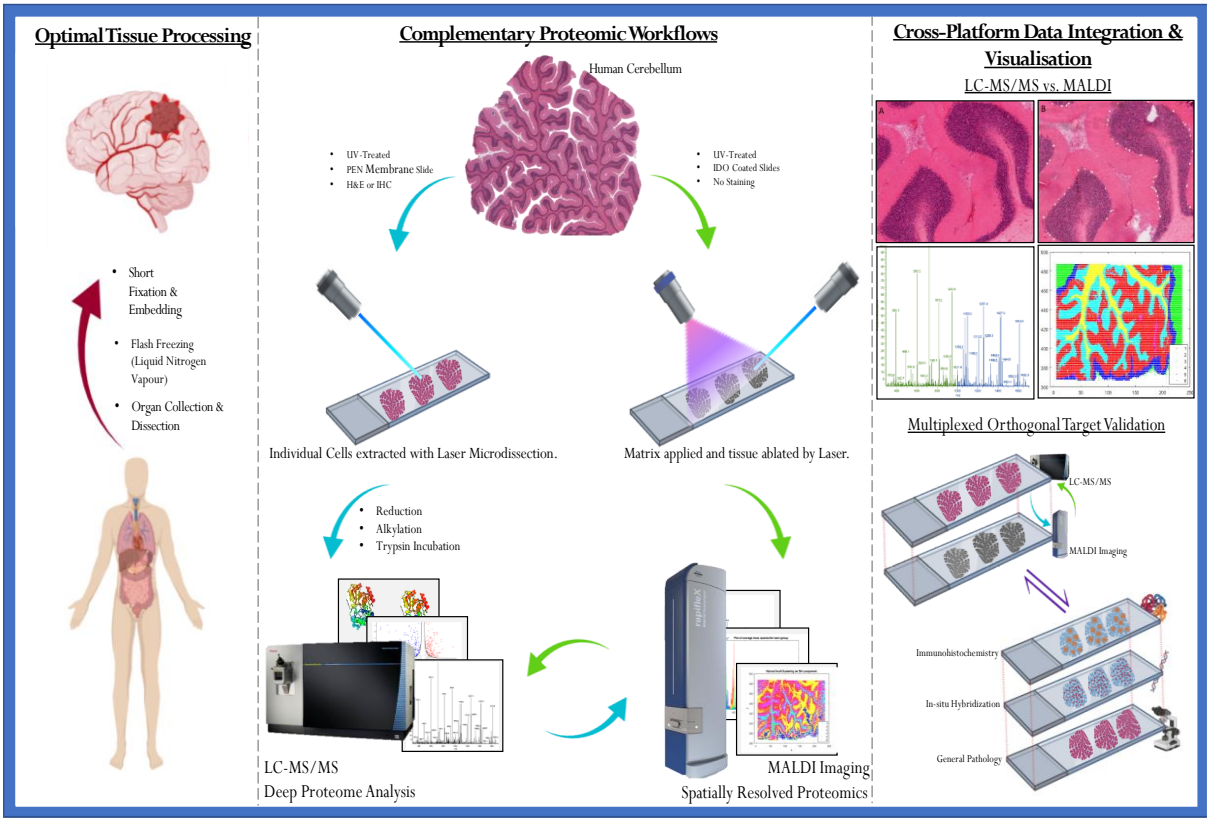
Project 4

Project Title: Spatially resolved 3D mass spectrometry for cancer analytics in the human brain

Primary Supervisor: Roman Fischer - roman.fischer@ndm.ox.ac.uk
Additional Supervisor: Olaf Ansorge - olaf.ansorge@ndcn.ox.ac.uk

Project Overview

'Oncometabolomics' links metabolic cancer signatures to genetic subtypes of primary and metastatic brain cancers. For example, glioblastomas (GBMs) may be associated with D-2-hydroxyglutarate (2HG) (1, 2). Although the mutations leading to cancer associated phenotypes are often known, there are no data on the spatially resolved proteomic context that is driving oncogenesis at the molecular level. To resolve this missing link, we have developed a spatial proteomics workflow to contribute the first integrated three-dimensional 'oncomap' of GBM. Specifically, we will use laser-capture microscopy (LCM)-derived samples for ultra-deep LC-MS/MS analysis (3) in the three-dimensional context of human brain tissue. This technology is to be integrated with mass spectrometric tissue imaging (MSI) using MALDI (4). The project will use resection specimens of human GBM, serially sectioned for three-dimensional reconstruction and integration of digital microscopy, metabolomics, proteomics and potentially transcriptomics (Figure). Our project focusses on the transition from already established 2-dimensional workflows into 3-dimensional space and the generation of the first 3-dimensional proteomic map of human glioblastoma derived tissue (5).



Integrated Proteomic Imaging Workflow. Pilot data leading to this application using human brain (cerebellum). Pictures are actual data. See also our paper Davis et al. (3). **Left:** We have developed a novel liquid nitrogen vapour (LNV) freezing method for large specimens and a complementary short-fixation protocol. Both are ideal for MSI. **Centre:** Left workflow – laser capture dissection of cerebellar Purkinje cells for "deep" LC-MS/MS. M/z peaks displayed. Right

workflow: MALDI MSI of a serial section of the same tissue. MALDI image displayed. **Right:** Top – integration of LC-MS/MS and MALDI MSI datasets. Bottom – multiplex immunohistochemical validation of novel targets at (sub-)cellular resolution (using existing Perkin Elmer / Codex platforms at the University of Oxford).

Training opportunities

The candidate will acquire highly transferable skills in mass spectrometry, proteomics, metabolomics, data analytics/integration and oncometabolomics:

- Multidisciplinary training in the 'final frontier' technologies of tissue 'omics', which is thought to disrupt diagnostic pathology in the next decade.
- Training on key global health priority areas: "new technologies and infrastructure", "precision medicine", "discovery science" with a focus on an area of unmet need: neuro-oncology and neurodegeneration (CRUK and MRC Neurosciences and Mental Health Board priorities).
- Direct access and training on state-of-the-art key technologies/equipment such as laser capture microdissection, high throughput LC-MS/MS, MALDI imaging (pending)
- Multi-omic data integration and visualisation (Collaboration with Big Data Institute)

Supervisors

Prof Roman Fischer

I am a principal investigator for the TDI/NDM and the director of the Discovery Proteomics Facility, which operates as a research facility within the TDI MS Lab under my supervision, supporting researchers across Oxford the UK, Europe, Africa and the US. Due to Oxford's unique position as a world-leading Institution in biomedical and global health research, including access to large biobanks and other sample repositories, there is an increasing demand for large-scale clinical proteomic workflows to detect drug targets and molecular markers of disease. The DPF is highly successful, having contributed to a large body of publications and is one of the leading facilities of its kind in the UK.

Besides 10s of collaborations covering many basic science and clinical projects, my own research interests focus on method development and optimisation of proteomics workflows to boost results from limited sample amounts, as demonstrated by Gel-Aided-Sample-Preparation (GASP) and the generation of one of the most comprehensive cancer proteomes published so far.

I am developing high-throughput/automated sample handling for clinical proteomics and to allow the detection of deep proteomes from single cell clusters, isolated by laser capture micro dissection (LCM). This technique allows the detection of up to 3000 proteins from 100-200 individual cells of the same type and vicinity (Purkinje cells). The combination of these techniques with higher throughput workflows will allow a deep characterisation of protein expression profiles with cell phenotype resolution (3D proteome map of a biological structure such as a tumour).

In the Discovery Proteomics Facility of the Target Discovery Institute we provide advice in experimental design, sample preparation, sample analysis with state-of-the-art LCMS workflows and data analysis to researchers from Oxford University and national and international collaborators. We routinely use label-free quantitation, SILAC, TMT, SWATH and other methodologies on diverse samples (i.e. cells, tissues, immuno precipitates et al.) and have developed sample preparation techniques to access the deep proteome form little sample amounts using instrumentation such as TimsTOF Pro, Orbitrap Fusion Lumos or Q-Exactive HF.

Prof Olaf Ansorge
I am a practising consultant neuropathologist, the director of the Oxford Brain Bank, funded by the MRC, and lead for neuropathology, genomics and biobanking of the forthcoming GBP5.8 million "Tessa Jowell BRAIN-MATRIX" trial, which aims, for the first time, to establish a state-of-the-art tissue diagnostic infrastructure for improved brain tumour diagnostics in the UK. It comprises 10 UK centres, with Oxford acting as the molecular diagnostics hub. The ISCF and Genomics England are funding whole genome and epigenome sequencing for all recruited individuals (n=1500 over five years, start date 2020). To be able to perform MSI on a well-defined subset of these patient samples represents an ideal opportunity, as MSI data must be understood in the genetic, epigenetic and neuropathological context. Partnership with Philips Healthcare, provider of digital pathology solutions for Oxford (also ISCF funded), further enhances this studentship as it will provide the basis for digital integration of structural and MSI data on a pixel-to-pixel basis.

In support of my role as PI for this studentship I would like to cite a previous, successfully completed, DPhil project that I have led in partnership with Renishaw UK PLC, leader in Raman spectroscopy, and collaborators in Chemistry. This has resulted in the development of a Raman-spectroscopy based model for the distinction of two of the main subtypes of human gliomas, with potential application as an intraoperative neurosurgical probe. This was funded by a CRUK clinical studentship (three years).

Finally, my proposed Co-supervisor, Roman Fischer and I are jointly supervising two students who have developed a laser-capture microscopy (LCM) based "microproteomics" workflow (REF 3). LCM proteomics and MSI are highly complementary, as generally the LCM-proteomics approach can be maximised for depths of proteome analysis, whilst MSI can be maximised for spatial resolution.

My overall professional aim is to improve brain tissue diagnostics for precision medicine by working with academic and commercial basic scientists; these collaborations are the most satisfying aspect of my professional life.

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2. Yong C, Stewart GD, Frezza C. Oncometabolites in renal cancer. Nat Rev Nephrol. 2019.

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4. Niehaus M, Soltwisch J, Belov ME, Dreisewerd K. Transmission-mode MALDI-2 mass spectrometry imaging of cells and tissues at subcellular resolution. Nat Methods. 2019;16(9):925-31.

5. Rozenblatt-Rosen O, Regev A, Oberdoerffer P, Nawy T, Hupalowska A, Rood JE, et al. The Human Tumor Atlas Network: Charting Tumor Transitions across Space and Time at Single-Cell Resolution. Cell. 2020;181(2):236-49.

Project 5-1

Project Title: Gene editing and base editing to cure severe forms of alpha thalassaemia

Primary Supervisor: Doug Higgs - doug.higgs@imm.ox.ac.uk
Additional Supervisors: Christian Babbs - christian.babbs@imm.ox.ac.uk; James Davies - james.davies@imm.ox.ac.uk

Project Overview
Thalassaemia is the most common form of inherited anaemia found throughout the world and one of the most common single gene disorders. In all cases, it results from an imbalance in the production of the α-like and β-like globin chains of haemoglobin (Hb), leading to α-thalassaemia and β-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 how mammalian genes are normally switched on and off during erythropoiesis and identifying many general principles underlying human molecular genetics.

Alpha thalassaemia is particularly common in southeast Asia, including southern China. The two most severe forms of α-thalassaemia cause HbH disease and the Hb Bart's Hydrops Fetalis Syndrome (BHFS). HbH disease is associated with a moderate or severe anaemia which may require regular blood transfusion. BHFS causes lethal neonatal anaemia and without intensive care and blood transfusion or bone marrow transplantation such infants die in the third trimester of pregnancy or shortly after birth. Our laboratory has defined most of the common mutations associated with α-thalassaemia. Whereas normal individuals have four α-genes (αα/αα), those with HbH disease inherit just one functional α-gene (--/-α), and those with HBFS inherit no functional α-genes (--/--).

To ameliorate or even cure these conditions we are focussed on two approaches. The first is simply to replace the missing α-genes in their normal location on human chromosome 16 using CRISPR-based site-directed genome editing. In this way the newly inserted α-genes would be activated by the α-globin superenhancer, which remains intact upstream of the deletion most commonly found in infants with BHFS: the so called southeast Asian deletion ($--^{SEA}$). Pre-clinical studies to develop this protocol will use the well-defined HUDEP2 cell line, and primary human $CD34^+$ progenitor cells, both of which can be differentiated to produce normal red blood cells.

The second approach involves reactivating the embryonic α-like gene (the zeta [ζ] gene) which remains intact but is silenced in the $--^{SEA}$ allele. Previous work has shown that embryonic Hb (HbPortland II: $\zeta_2\beta_2$) would functionally complement the missing adult Hb (HbA: $\alpha_2\beta_2$). Current work in the laboratory has identified some key pathways that silence ζ-globin expression and future work is aimed at identifying the *cis*- and *trans*-acting elements through which these pathways exert their effects. This in turn will allow us to develop CRISPR and base editing approaches to abrogate these silencing pathways and de-repress ζ-globin expression to therapeutically useful levels.

The successful applicant will join a lab of approximately 14 including students, post-docs and research assistants. The project would be suitable for a clinician-scientist or a basic scientist.

Training Opportunities (both Laboratory and clinical)
These projects will involve all techniques associated with current molecular and cell biology to study transcriptional and epigenetic programmes and the 3-D structure of the genome. In addition, we routinely use genome editing with programmable nucleases and base editing. Students will use state-of-the-art flow sorting and imaging to isolate and study specific populations of haematopoietic cells. Many studies will involve the analysis of chromatin and transcription in single cells. All students will receive training in computational biology. The scientific laboratories work in collaboration with one of the largest centres of haematology in the UK and collaborate with many international groups with an interest in thalassaemia.

Supervisor
Douglas Higgs (FRS, DSc, FRCP, FRCPath FMedSci, member of EMBO) qualified in Medicine at King's College Hospital Medical School (University of London) in 1974 and trained as a haematologist. He joined the MRC Molecular Haematology Unit (Oxford) in 1977 and is currently Professor of Molecular Haematology at the University of Oxford, honorary consultant in the Department of Clinical Haematology (ORHA). Until recently he was Director of the MRC Molecular Haematology Unit (MHU) and Director of the Weatherall Institute of Molecular Medicine (WIMM). The main interest of his laboratory is to understand how mammalian genes are switched on and off during differentiation and development using haematopoiesis as the experimental model. The laboratory also aims to use this knowledge to cure human genetic diseases. His laboratory has identified many of the principles underlying human genetic disease via their studies on thalassaemia and they have made considerable contributions to the field of mammalian gene regulation.

http://www.imm.ox.ac.uk/doug-higgs

Key publications

1. King AJ, Songdej D, Downes DJ, Beagrie RA, Liu S, Buckley M, Hua P, Suciu MC, Marieke Oudelaar A, Hanssen LLP, Jeziorska D, Roberts N, Carpenter SJ, Francis H, Telenius J, Olijnik AA, Sharpe JA, Sloane-Stanley J, Eglinton J, Kassouf MT, Orkin SH, Pennacchio LA, Davies JOJ, Hughes JR, **Higgs DR**, Babbs C. Reactivation of a developmentally silenced embryonic globin gene. Nat Commun. 2021 Jul 21;12(1):4439. doi: 10.1038/s41467-021-24402-3. PMID: 34290235; PMCID: PMC8295333.

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Project 5-2

Project Title: Understanding how superenhancers regulate gene expression

Primary Supervisor: Doug Higgs - doug.higgs@imm.ox.ac.uk
Additional Supervisor: Mira Kassouf - mira.kassouf@imm.ox.ac.uk

Project Overview
Key events in cell biology, including lineage-specification, commitment to differentiation and cell maturation can be attributed primarily to changes in gene expression. Such changes are ultimately mediated by transcription factors (TFs) and co-factors (Co-Fs) binding to enhancers (~200-800bp of nucleosome free DNA) and acting upon promoters located 10s-1000s kb away to modulate their patterns of expression in time and space. These molecular switches provide the fundamental units within gene regulatory circuits and it is now clear that variation and perturbation of enhancers underlie many human traits and predisposition to common diseases.

Although enhancers were first identified almost 40 years ago, the mechanisms by which they activate gene expression are still not understood. Today, sequences with the chromatin signature of an enhancer can be easily identified genome-wide but their functional role in vivo cannot be easily predicted. The mechanisms underlying enhancer function are further complicated by the fact that individual genes may be regulated by many enhancers acting in concert. The first example of such complex enhancers containing multiple individual enhancer elements working together to control gene expression was found in the β-globin complex. This cluster of enhancers was referred to as a locus control region (LCR). Subsequently, such regions have been referred to as superenhancers and these elements are particularly found associated with critical genes that determine cell fate.

Like the β-globin cluster, the α-globin cluster is controlled by five enhancers which together form a superenhancer to activate the α-globin genes in erythroid cells, and we use the β-globin gene cluster as a model to understand the general principles by which such elements activate gene expression. We have previously deleted the enhancer elements individually and in informative combinations to analyse the contribution of each element to α-globin gene expression. This initially suggested that each element contributed in an additive manner. More recently we have been sequentially rebuilding the superenhancer from 0-5 elements and this shows that, in fact, the enhancers work in a more complex synergistic manner. Importantly, two elements that appear to have no intrinsic activity play an important role in enabling the full effect of the conventional enhancers.

Using well established DNA synthesis and gene editing protocols, combined with a wide range of molecular and imaging analyses, we are now investigating the effects of proximity, orientation and sequence of the superenhancer elements to understand exactly how they interact with their cognate promoter to enhance transcription. Our aim is to understand the general principles by which enhancers regulate gene expression.

The successful applicant will join a lab of approximately 14 including students, post-docs and research assistants. The project would be suitable for a clinician-scientist or a basic scientist.

Training Opportunities (both Laboratory and clinical)
These projects will involve techniques associated with current molecular and cell biology to study transcriptional and epigenetic programmes, and the 3-D structure of the genome. In addition, we routinely use cell culture systems as well as mouse models combined with genome editing with programmable nucleases to manipulate and assess the effect of the elements in question. Students will use state-of-the-art cellular labelling followed by flow sorting and imaging techniques to isolate and study specific populations of haematopoietic cells. Many studies will involve the analysis of chromatin and transcription in single cells. All students will receive training in computational biology. The scientific laboratories work in collaboration with one of the largest centres of haematology in the UK and collaborate with many international groups with an interest in thalassaemia.

Supervisor
Douglas Higgs (FRS, DSc, FRCP, FRCPath FMedSci, member of EMBO) qualified in Medicine at King's College Hospital Medical School (University of London) in 1974 and trained as a haematologist. He joined the MRC Molecular Haematology Unit (Oxford) in 1977 and is currently Professor of Molecular Haematology at the University of Oxford, honorary consultant in the Department of Clinical Haematology (ORHA), Director of the MRC Molecular Haematology Unit (MHU) and Director of the Weatherall Institute of Molecular Medicine (WIMM). The main interest of his laboratory is to understand how mammalian genes are switched on and off during differentiation and development using haematopoiesis as the experimental model. His laboratory has identified many of the principles underlying human genetic disease via their studies on thalassaemia and they have made considerable contributions to the field of mammalian gene regulation.

http://www.imm.ox.ac.uk/doug-higgs

Key publications

1. Hay D, Hughes JR, Babbs C, Davies JOJ, Graham BJ, Hanssen L, Kassouf MT, Marieke Oudelaar AM, Sharpe JA, Suciu MC, Telenius J, Williams R, Rode C, Li PS, Pennacchio LA, Sloane-Stanley JA, Ayyub H, Butler S, Sauka-Spengler T, Gibbons RJ, Smith AJH, Wood WG, **Higgs DR**. Genetic dissection of the α-globin super-enhancer in vivo. Nat Genet. 2016 Aug;48(8):895-903. doi: 10.1038/ng.3605. Epub 2016 Jul 4. PMID: 27376235; PMCID: PMC5058437.

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3. Hua P, Badat M, Hanssen LLP, Hentges LD, Crump N, Downes DJ, Jeziorska DM, Oudelaar AM, Schwessinger R, Taylor S, Milne TA, Hughes JR, Higgs DR, Davies JOJ. Defining genome architecture at base-pair resolution. Nature. 2021 Jul;595(7865):125-129. doi: 10.1038/s41586-021-03639-4. Epub 2021 Jun 9. PMID: 34108683.

4. Oudelaar AM, Higgs DR. The relationship between genome structure and function. Nat Rev Genet. 2021 Mar;22(3):154-168. doi: 10.1038/s41576-020-00303-x. Epub 2020 Nov 24. PMID: 33235358.

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Project 6

Project Title: Cardiac neurobiology of arrhythmia in human cell models: novel therapeutic target

Primary Supervisor: David Paterson - david.paterson@dpag.ox.ac.uk
Additional Supervisor: Dan Li - dan.li@dpag.ox.ac.uk

Project Overview

Heightened sympathetic drive (dysautonomia) is a hallmark of several cardiovascular diseases including SARS-CoV-2. It is also a powerful prognostic predictor for arrhythmia and sudden cardiac death, especially in patients with channelopathies (long QT syndrome-LQTS, and catecholaminergic polymorphic ventricular tachycardia-CPVT). However, little is known about the molecular targets underlying this dysautonomia. We recently discovered that human and rat cardiac sympathetic neurons have their own ACE2 coupled renin angiotensin II pathway that is linked to abnormal neurotransmission. These neurons drive cardiac excitability and can increase the propensity for life-threatening arrhythmias. We have identified a novel pathway using a combination of single cell and bulk RNAseq, neurochemistry, FRET imaging and single cell electrophysiology. This pathway involves impairment of cyclic nucleotide coupled phosphodiesterases (PDE) linked to enhanced intracellular calcium transients and exocytosis from rat sympathetic neurons. In particular, the adaptor protein Nos1-ap, Pde2A, and Ace2 are associated with sympathetic hyperexcitability. These proteins are also conserved in human stellates from patients with CPVT, although their role in neuronal-myocyte cellular function is unknown.

In this project, firstly, we will derive sympathetic neurons and cardiomyocytes from human pluripotent stem cells (hPSC) from healthy people and CPVT patient, obtain a comprehensive understanding of the molecular, cell biological and functional identities characteristic via single cell RNAseq, qPCR, IF staining, calcium imaging and measure cyclic nucleotide using Fluorescence resonance energy transfer (FRET), comparison these phenotypes with primary cultured sympathetic neurons and cardiomyocyte. Secondly, we will co-culture or cross culture these hPSC-derived sympathetic neurons and cardiomyocytes, to test the utility of this intervention on hiPSCs to modulate neuronal driven cardiac excitability, to determine their role in driving abnormal transmission and arrhythmia. This will provide a novel and valuable evidence of stem cell therapeutic mechanisms for treatment of autonomic dysfunction like CPVT.

Training opportunities

The student would receive training in Human Pluripotent Stem Cells (iPSC) culture and differentiation; Cell physiology (patch clamp, FRET, Ca^{2+} imaging, electrical mapping); Neurochemistry; Molecular biology (RNA sequencing); Immunohistochemistry (human tissue). In addition, the student would have access to a wide-range of seminars and training opportunities through the many research institutes and centres based in Oxford, and would attend relevant conferences at domestic and international.

Supervisors

David Paterson is a Head of Department and leads a research team in the area of cardiac neurobiology. They are interested in how both branches of the cardiac autonomic nervous system communicate at the end organ level and whether oxidative stress plays a role in uncoupling pre-synaptic and post synaptic signalling. The endogenous gas nitric oxide is now thought to be a key

intermediary in cardiac inter/intracellular signalling, where it has been shown to regulate several ion channels that control cardiac excitability. His group has developed a method for targeting the enzyme involved in making nitric oxide using a gene transfer approach involving cell specific viral vectors to study the physiology of this messenger in normal and diseased hearts. Recently his group derived sympathetic neurons from human pluripotent stem cells with defined conditions from healthy people and CPVT disease patient. https://www.dpag.ox.ac.uk/team/david-paterson

Dan Li is a University research lecturer in the Department of Physiology, Anatomy and Genetics. Her main research interest is focused on understanding how the autonomic nervous system influences cardiac function in health and disease. She developed a primary culture system of sympathetic ganglion neurons, using cellular and molecular approaches to investigate the interactions between different signalling cascades (Ca2+, nitric oxide, natriuretic peptide, cAMP, cGMP and other) in the cytosol and the sub-organelles (mitochondria and endoplasmic reticulum). She utilises several genetically encoded fluorescent biosensors for monitoring second messengers using fluorescence microscopy and Fluorescence Resonance Energy Transfer (FRET) in living cells to understanding how these second messengers impact on neuronal and cardiac function.
https://www.dpag.ox.ac.uk/team/dan-li

Clinical supervisors
Associate Prof. Neil Herring, Consultant cardiologist

Key publications

1. Davis H, Herring N, Paterson DJ. Downregulation of M Current is Coupled to Membrane Excitability in Sympathetic Neurons Before the Onset of Hypertension. (2020) Hypertension Dec 76 (6), 1915-1923.
2. Bardsley EN, Neely OC, Paterson DJ (2020). Angiotensin Peptide Synthesis and Cyclic Nucleotide Modulation in Sympathetic Stellate Ganglia. J Mol Cell Cardiol. 2020 Jan 10. pii: S0022-2828(19)30385-2.
3. Herring N, Kalla M, Paterson DJ (2019) The Autonomic Nervous System and Cardiac Arrhythmias: Current Concepts and Emerging Therapies. Nat Rev Cardiol 2019 June 13 doi: 10.1038/s41569-019-0221-2
4. Li D. and Paterson DJ., (2019), Semin Cell Dev Biol. Pre-synaptic sympathetic calcium channels, cyclic nucleotide-coupled phosphodiesterases and cardiac excitability. Semin Cell Dev Biol. doi: 10.1016/j.semcdb.2019.01.010
5. Liu K, Li D, Hao G, McCaffary D, Neely O, Woodward L, Ioannides D, Lu CJ, Brescia M, Zaccolo M, Tandri H, Ajijola OA, Ardell JL, Shivkumar K, Paterson DJ. (2018) Phosphodiesterase 2A as a therapeutic target to restore cardiac neurotransmission during sympathetic hyperactivity. JCI Insight. doi: 10.1172/jci.insight.98694

Project 7-1

Project Title: SARS-CoV-2 replication, assembly, and egress

Primary Supervisor: Peijun Zhang - peijun.zhang@strubi.ox.ac.uk

Project Overview

The ongoing global pandemic of coronavirus disease 2019 (COVID-19) resulted from the outbreak of SARS-CoV-2 in December 2019. Currently, multiple efforts are being made to rapidly develop vaccines and treatments to fight COVID-19. Understanding the SARS-CoV-2 infection process in human cells is critical to such efforts in vaccine development and therapeutic treatment. Yet, our currently knowledge is largely based on the previous coronaviruses, very little is known about cellular structural details of SARS-CoV-2 infection and virus-host interactions. In this project, we will use a correlative multi-scale imaging approach to dissect the individual steps during SARS-CoV-2 infection, namely the genome replication, the virus assembly and egress, within the native cells. The replication of SARS-CoV-2 is a complicated multistage process that involves several different cellular compartments and the activity of many viral and cellular proteins. We will employ cutting-edge cryoEM/cryoET and cryoFIB/SEM imaging technologies to reveal the mechanisms of SARS-CoV-2 replication, from the whole 3D volume of infected cells by serial cryoFIB/SEM method to the structures of individual viral and host protein complexes involved in SARS-CoV-2 replication at subnanometer or near-atomic resolutions by cryoEM/ET. Integrating such multi-scale structural information will provide essential knowledge of virus and host interplay that will not only help to fight COVID-19, but also have a broader impact on preventing and combating future emergence of other viruses.

Training opportunities

We are located in the Division of Structural Biology, Wellcome Trust Centre for Human Genetics, which provides an ideal environment for multidisciplinary and integrative studies. We also have regular access to eBIC at Diamond Light Source for data collection and computation. Individual projects are tailored to particular student's interests and cover techniques in molecular, cellular and structural biology. Through the projects, students will be trained in

- Molecular cloning, protein expression and protein purification
- Protein biochemical/biophysical characterization
- CryoEM single particle structure determination and /or
- Cryo-electron tomography and sub-tomogram averaging
- Correlative light and cryoEM imaging of virus infection
- Cryo-focused ion beam and cryo-SEM volume imaging
- Data analysis and image reconstruction
- Computer molecular dynamics simulations

Supervisor

Professor Peijun Zhang is a Professor of Structural Biology and a Wellcome Trust Investigator at the Nuffield Department of Medicine at Oxford University. She obtained her Ph.D. in Biophysics and Physiology from University Virginia, M.S. in Solid State Physics and B.S. in Electrical Engineering from Nanjing University, China. She was a post-doctoral fellow and subsequently a staff scientist at the National Cancer Institute, NIH, and was recruited as an Assistant Professor in 2006 and achieved a tenured Associate Professor in 2012 at the University of Pittsburgh School of Medicine.

She was recruited to Diamond Light Source in 2016 as the founding director of eBIC (the UK National Electron Bio-imaging Centre) and jointly as a Professor at the University of Oxford. Professor Zhang's research focuses on the molecular mechanisms of host and pathogen interactions, including SARS-CoV-2, HIV-1 and pathogenic bacteria, by developing and combining novel technologies for high-resolution cryoEM and cryoET. She received many awards, including "Carnegie Science Emerging Female Scientist Award", The University of Pittsburgh Senior Vice Chancellor's Award, and the "Wellcome Trust Investigator Award". She has supervised 27 Postdocs and 10 PhD students.

Key publications

1. Mendonça L, Howe A, Gilchrist JB, Sheng Y, Sun D, Knight ML, Zanetti-Domingues LC, Bateman B, Krebs AS, Chen L, Radecke J, Li VD, Ni T, Kounatidis I, Koronfel MA, Szynkiewicz M, Harkiolaki M, Martin-Fernandez ML, James W, **Zhang P*** (2021) Correlative multi-scale cryo-imaging unveils SARS-CoV-2 assembly and egress. *Nat Commun*. 12(1):4629.

2. Watanabe Y, Mendonça L, Allen E, Howe A, Lee M, Allen J, Chawla H, Pulido D, Donnellan F, Davies H, Ulaszewska,M, Belij-Rammerstorfer S, Morris S, Krebs A, Dejnirattisai W, Mongkolsapaya J, Supasa P, Screaton G, Green C, Lambe T, **Zhang P***, Gilbert S, Crispin M (2021) Native-like SARS-CoV-2 spike glycoprotein expressed by ChAdOx1 nCoV-19 vaccine. *ACS Central Science* Article ASAP DOI: 10.1021/acscentsci.1c00080

3. Liu C, Mendonça L, Yang Y, Gao Y, Shen C, Liu J, Ni T, Ju B, Liu C, Tang X, Wei J, Ma X, Zhu Y, Liu W, Xu S, Liu Y, Yuan J, Wu J, Liu Z, Zhang Z, Liu L, Wang P, **Zhang P*** (2020) The Architecture of Inactivated SARS-CoV-2 with Postfusion Spikes Revealed by CryoEM and CryoET. *Structure* 28(11):1218-1224

4. Ni T, Gerard S, Zhao G, Dent K, Ning J, Zhou J, Shi J, Anderson-Daniels J, Li W, Jang S, Engelman AN, Aiken C, **Zhang P*** (2020) Intrinsic curvature of HIV-1 CA hexamer underlies capsid topology and interaction with cyclophilin A. *Nat Struct Mol Biol* **27,** 855–862.

5. Zhao G., Perilla J.R., Yufenyuy E.L., Meng X., Chen B., Ning J., Ahn J., Gronenborn A.M., Schulten K.*, Aiken C.* and **Zhang P.*** (2013) Mature HIV-1 Capsid Structure by Cryo-electron Microscopy and All-atom Molecular Dynamics. *Nature* 497(7451

Project 7-2

Project Title: Imaging HIV-1 nuclear import by in situ cryo-tomography and correlative microscopy

Primary Supervisor: Peijun Zhang - peijun.zhang@strubi.ox.ac.uk

Project Overview

Human immunodeficiency virus type 1 (HIV-1) is the causative agent behind acquired immunodeficiency syndrome (AIDS) that currently has no cure or vaccine. While antiviral treatments are effective, the rise of drug-resistant strains has become a growing concern. HIV-1 primarily infects the immune system, targeting CD4+ T cells and macrophages and is a lentivirus known to be able to infect non-dividing cells, requiring it to exploit nuclear import mechanisms. This process is dependent on the viral capsid. The HIV capsid is a conical structure that houses the genomic material of the virus. It needs to be metastable in order be protective while allowing timely disassembly (termed uncoating) to release its genome. The dynamics of the capsid nuclear import and uncoating are still known and is modulated by host-dependency and restriction factors.

We aim to apply multi-imaging modalities to investigate uncoating and nuclear import of HIV. These will include super-resolution fluorescence microscopy (including the newest MINFLUX system), Focused Ion Beam and Scanning electron microscopy (cryoFIB/SEM), cryo-electron microscopy and cryo-electron tomography (cryoEM/ET). The viral core and host factors will be fluorescently tagged using non-natural AA and click chemistry and infection will be monitored from viral attachment to nuclear import. The sample will be cryo-preserved and imaged by cryoEM/ET and cryoFIB/SEM. The combination of these imaging techniques will yield unparallel structural information of the HIV infection process within the native cells, providing the framework for development of novel therapeutics targeting HIV infection in the future.

Training opportunities

We are located in the Division of Structural Biology, Wellcome Trust Centre for Human Genetics, which provides an ideal environment for multidisciplinary and integrative studies. We also have regular access to eBIC at Diamond Light Source for data collection and computation. Individual projects are tailored to particular student's interests and cover techniques in molecular, cellular and structural biology. Through the projects, students will be trained in

- Molecular cloning, protein expression and protein purification
- Protein biochemical/biophysical characterization
- CryoEM single particle structure determination and /or
- Cryo-electron tomography and sub-tomogram averaging
- Correlative light and cryoEM imaging of virus infection
- Cryo-focused ion beam and cryo-SEM volume imaging
- Data analysis and image reconstruction
- Computer molecular dynamics simulations

Supervisor

Professor Peijun Zhang is a Professor of Structural Biology and a Wellcome Trust Investigator at the Nuffield Department of Medicine at Oxford University. She obtained her Ph.D. in Biophysics and Physiology from University Virginia, M.S. in Solid State Physics and B.S. in Electrical Engineering from Nanjing University, China. She was a post-doctoral fellow and subsequently a staff scientist at

the National Cancer Institute, NIH, and was recruited as an Assistant Professor in 2006 and achieved a tenured Associate Professor in 2012 at the University of Pittsburgh School of Medicine. She was recruited to Diamond Light Source in 2016 as the founding director of eBIC (the UK National Electron Bio-imaging Centre) and jointly as a Professor at the University of Oxford. Professor Zhang's research focuses on the molecular mechanisms of host and pathogen interactions, including HIV-1, SARS-CoV-2 and pathogenic bacteria, by developing and combining novel technologies for high-resolution cryoEM and cryoET. She received many awards, including "Carnegie Science Emerging Female Scientist Award", The University of Pittsburgh Senior Vice Chancellor's Award, and the "Wellcome Trust Investigator Award". She has supervised 27 Postdocs and 10 PhD students.

Key publications

1. Mendonça L, Howe A, Gilchrist JB, Sheng Y, Sun D, Knight ML, Zanetti-Domingues LC, Bateman B, Krebs AS, Chen L, Radecke J, Li VD, Ni T, Kounatidis I, Koronfel MA, Szynkiewicz M, Harkiolaki M, Martin-Fernandez ML, James W, **Zhang P*** (2021) Correlative multi-scale cryo-imaging unveils SARS-CoV-2 assembly and egress. *Nat Commun*. 12(1):4629.

2. Ni T, Gerard S, Zhao G, Dent K, Ning J, Zhou J, Shi J, Anderson-Daniels J, Li W, Jang S, Engelman AN, Aiken C, **Zhang P*** (2020) Intrinsic curvature of HIV-1 CA hexamer underlies capsid topology and interaction with cyclophilin A. *Nat Struct Mol Biol* **27,** 855–862.

3. Zhao G., Perilla J.R., Yufenyuy E.L., Meng X., Chen B., Ning J., Ahn J., Gronenborn A.M., Schulten K.*, Aiken C.* and **Zhang P.*** (2013) Mature HIV-1 Capsid Structure by Cryo-electron Microscopy and All-atom Molecular Dynamics. *Nature* 497(7451):643-6. Featured on the cover of Nature.
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Project 8

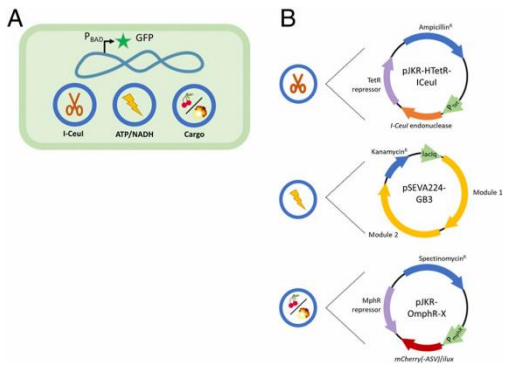
Project Title: SimCells – characterising a novel platform for targeted immunotherapy

Primary Supervisor: Eileen Parkes - eileen.parkes@oncology.ox.ac.uk
Additional Supervisor: Wei Huang - wei.huang@eng.ox.ac.uk

Project overview

SimCells are a novel platform, developed by Professor Wei Huang, for the delivery of anti-cancer therapeutics (Fan et al. 2020). SimCells are chromosome-free bacteria, which can be genetically programmed to express a protein of choice (*Figure 1*). These bacteria are non-reproducing and can therefore be safely targeted to the tumour site, expressing surface nanobodies to both target cancer cells and crosslink anti-cancer T cells to the tumour cells and promote cell killing. This novel drug-delivery platform carries huge potential for delivery of almost any antibody or enzymatic anti-cancer treatment that SimCells are produce. As this therapeutic development is in its infancy, the proposed project offers an unparalleled opportunity to contribute to the development and translation of this treatment to a preclinical stage, with clinically focused studies characterising this platform using *in vivo* and *ex vivo* (using patient samples) models of cancer.

Figure 1A) An example of a constructed SimCell, with chromosomal expression of GFP, and three additional plasmids used to degrade the chromosome, reintroduce glycolysis and produce protein. B) Details of introduced plasmids, involved in chromosomal degradation, energy production (via glycolysis) and a further inducible expression vector.



In this proposal, the candidate will generate novel mini- and standard SimCells capable of targeting specific tumour antigens. Using the syngeneic immunocompetent *in vivo* models of cancer, the candidate will generate cancer antigen-targeting SimCells, for example, gp70, also expressing a CD3 antibody. Bacteria are uniquely suited to the tumour microenvironment, adapted to the acidic and hypoxic conditions typical of tumours. As such, SimCells represent an ideal platform for intratumoral immune priming and activation to enhance immunotherapeutic responses. SimCells are a low-risk therapeutic approach – although they can be endlessly generated from engineered parent cells, they have no reproductive capability and therefore dosage and therapeutic activity can be tightly controlled.

Aims:

(1) Optimisation of SimCells as a therapeutic approach using in vivo models

Using syngeneic models of murine cancer, including in-house DNA repair deficient models of breast and prostate cancer, the candidate will optimise the therapeutic approaches using SimCells targeting existing known immune modulating antibodies such as anti-PD-1/L1 and anti-CTLA4.

Mode of administration, scheduling of treatment and pharmacokinetic studies will be performed to develop effective pre-clinical therapeutic regimens.

(2) Discovery of novel targets for use in SimCells

Given the unlimited potential of SimCells to express a desired protein, the role of SimCells in producing enzymes capable of modulating the immuno-metabolic tumour microenvironment will be explored. This will include in vitro studies using normoxic and hypoxic conditions, representative of the tumour microenvironment. Using our previously characterised mouse models of DNA repair deficient cancer, the role of these novel targets in modulating immune responses, growth of primary tumour and metastatic growth will be defined.

(3) Combination therapies with existing immune checkpoint antibodies.

Combination treatments in vivo using SimCells expressing novel targets, in combination with existing immune checkpoint antibodies and intratumoural immune priming therapies, will be performed to identify the optimal schedule for a combination therapeutic approach.
Together, this innovative work will identify and optimise pre-clinical approaches using SimCells, identify novel immuno-metabolism modulating targets and define combination approaches for subsequent clinical translation.

Project 9-1

Project Title: Characterisation of monoclonal antibodies isolated from human B cells: implication in future SARS-CoV-2 variant vaccines

Primary Supervisor: Gavin Screaton - gavin.screaton@medsci.ox.ac.uk
Additional Supervisor: Juthathip Mongkolsapaya - jmongkol@well.ox.ac.uk

Project Overview
Reports of a severe acute respiratory syndrome in Wuhan China first appeared in December 2019. It was rapidly determined that coronavirus disease 2019 (COVID-19) was caused by infection with a novel beta coronavirus related to the SARS coronavirus and named SARS-CoV-2. SARS-CoV-2 spread rapidly leading to the global pandemic.

Since the first sequence of SARS-CoV-2 was deposited in early January 2020, viral genome sequencing efforts have been established in a number of countries to track the evolution of the virus. Coronaviruses are large positive strand RNA viruses and despite some proof-reading capacity, replication is intrinsically error prone. Progressive mutational change in the virus is therefore inevitable as it undergoes massive numbers of replicative cycles worldwide. In particular, changes were anticipated as the virus adapts to its new human host.

Many thousands of mutational changes have been described across the viral genome and whilst most will be detrimental or confer no advantage to the virus, some will be advantageous and be the subject of rapid natural selection. Mutations could confer advantage to the virus in a number of ways, but increased transmissibility or escape from innate or acquired immune responses are two potential examples.

Spike protein (S) is the major surface glycoprotein on coronaviruses. These characteristically trimeric spikes are subdivided into an N-terminal S1 domain responsible for attachment to host cells via its receptor ACE2 and a C-terminal S2 domain which is anchored in the viral membrane, cleaved from S1 following cellular attachment and responsible for membrane fusion and cell entry. S1 consists of an N-terminal domain (NTD) followed by the receptor binding domain (RBD) which mediates binding to ACE2, burying ~860 Å of surface area at its tip.

We have generated and characterised a large panel of monoclonal antibodies from SARS-CoV-2 infected individuals. We aim to study the antibodies, convalescent and vaccine sera to neutralize the variants and complement this with structural analyses of Fab/RBD complexes and map the antigenic space of current variants. This observation will provide important new insight for immunisation policy with future variant vaccines in populations.

Training opportunities
The student will join a team having expertise more than 20 years in virology, immunology and molecular biology. Our work has a great contribution in the field and successfully published in high impact journals including Cell, Nature, Science, Nature Medicine, Nature Immunology and Nature Communication with high citation. The student will be trained by experienced post-docs in a broad range of techniques such as basic virology (viral propagation, neutralisation and viral titration), immunology (ELISA, Immuno-precipitation, SDS-PAGE, Western blot, FPLC and affinity purification,

Flow cytometry, Single cell sorting, tissue/cell culture, molecular biology (PCR, using software programs to design primers, mutagenesis, deep sequencing of antibody repertoire, cloning, protein expression in bacteria, yeast, insect and mammalian cells systems) generating monoclonal antibodies from single human B cells. The project is in collaboration with investigators in the university and from national and international universities/institutes. Therefore, apart from meeting with wide range of experts in the campus, the student will have chances to interact/exchange knowledge with their scientists.

Supervisor: Professor Gavin Screaton
Co-Supervisor: Dr. Juthathip Mongkolsapaya
Professor Gavin Screaton is a Professor of Medicine, Head of the Medical Science Division, University of Oxford and Consultant Physician. He is a a Fellow of the Academy of Medical Sciences, a Fellow of the Royal College of Physicians, a member of the Association of Physicians. His research, which has been supported by a series of Fellowships awarded by the MRC, Wellcome Trust and European Union FP7 program, has covered a variety of topics from control of RNA processing and apoptosis to immunology. He is a Senior Investigator awarded by Wellcome Trust.

Dr. Juthathip Mongkolsapaya was trained in biochemistry, microbiology and immunology. Her work has been funded by Wellcome Trust, MRC, Newton-MRC and European Union FP7 program. The current interests of our laboratory revolve around the immunology of infectious diseases with a special interest in flavivirus and Corona virus infection, where our research is currently funded by the Wellcome Trust and MRC with active research collaborations in UK, South-East Asia, South Americas, and USA.

Key Publications

1. Dejnirattisai W., et al. The antigenic anatomy of SARS-CoV-2 receptor binding domain. Cell. 2021 Apr 15;184(8):2183-2200.e22. doi: 10.1016/j.cell.2021.02.032.
2. Supasa P., et al. Reduced neutralization of SARS-CoV-2 B.1.1.7 variant by convalescent and vaccine sera. Cell.2021 Apr 15;184(8):2201-2211.e7. doi: 10.1016/j.cell.2021.02.033.
3. Zhou D., et al. Evidence of escape of SARS-CoV-2 variant B.1.351 from natural and vaccine-induced sera. Cell. 2021 Apr 29;184(9):2348-2361.e6. doi: 10.1016/j.cell.2021.02.037.
4. Dejnirattisai W. et al. Antibody evasion by the P.1 strain of SARS-CoV-2. Cell. 2021 May 27;184(11):2939-2954.e9. doi: 10.1016/j.cell.2021.03.055.
5. Liu C., et al. Reduced neutralization of SARS-CoV-2 B.1.617 by vaccine and convalescent serum. Cell. 2021 Aug 5;184(16):4220-4236.e13. doi: 10.1016/j.cell.2021.06.020.

Project 9-2

Project Title: Development of a universal vaccine protecting from dengue and zika viruses infection

Primary Supervisor: Gavin Screaton - gavin.screaton@medsci.ox.ac.uk
Additional Supervisor: Juthathip Mongkolsapaya - jmongkol@well.ox.ac.uk

Project Overview

Viruses in the Flavivirus genus are the most important arthropod-borne human pathogens, causing increasingly serious epidemics such as the current zika virus (ZIKV) explosion in South America, for which neither preventive or curative treatments are available. Besides the current media impact of ZIKV, the Flaviviral disease that imposes the highest toll is dengue virus (DENV), caused by four viruses termed serotypes DENV1-4, which differ in amino acid sequence by 30-35%. The estimated global incidence is 390M cases per annum, of which 96M are clinically apparent, with around 25,000 deaths. Several factors drive the pandemic such as globalization, spread of the *Aedes* mosquito vector and inadequately planned urbanization. Dengue has caused explosive epidemics, which put huge stress on healthcare systems in endemic countries and although several dengue control strategies are being evaluated, it is generally agreed that an effective vaccine available to all age groups is required to make serious inroads into the burden of disease. Although Denvaxia, a first licensed dengue vaccine, provided a good efficacy, it increased a risk of develop severe disease in vaccinated seronegative individuals. This leads to imperative need to develop the better vaccine.

In the case of ZIKV, although discovered almost 70 years ago it is only recently that severe neurological sequelae including micocephaly and Guillain-Barré syndrome have been described. Not only both DENV and ZIKV co-circulate, they share the same vector ie Ades mosquito. Furthermore, their sequences are similar enough to induce cross reactive immune responses. We and others show a complex serological interaction between DENV and ZIKV. Anti-DENV antibodies can enhance ZIKV infection which may be one of factors contributing to the outbreak in Brazil where both virus co-circulate. There is now great pressure to produce a vaccine against ZIKV and improve the current licensed dengue vaccine, the extensive cross-reaction between DENV and ZIKV serologically must be considered in this regard. It is likely that the vaccine will need to be deployed in areas with high DENV seroprevalence and the difficulty of raising *de novo* ZIKV neutralizing responses in such a setting may be challenging. There is also the possibility that ZIKV vaccination in DENV naïve subjects may promote ADE of DENV and conversely that DENV vaccination may promote ADE of ZIKV infection. Over the past ten years, we have intensively studies hundreds of monoclonal antibodies recognising DENV and ZIKV resulting in identifying the characteristic of "good' and "bad" antibodies. In combination with crystal structures analysis, we characterized the part of viral envelop, epitope, recognised by highly potent neutralising antibodies which cross-reactive to all 4 dengue serotypes and ZIKV. We named the epitope as Envelope Dimer Epitope (EDE) (figure). The general aim of this project is to generate a soluble stable dimer version of the envelope and then through an iterative structural/modelling informed design process to develop immunogens to specifically target the generation of an anti-EDE response whilst resurfacing non-EDE related areas of the dimer to reduce the generation of less protective but infection enhancing antibodies. Immunogenicity will be tested in human immunoglobulin transgenic mice and *in vivo* neutralization will be tested in murine models of DENV and ZIKV infection. In conclusion, the project aim to generate a universal DENV/ZIKV vaccine which induces cross-protection for both viruses.

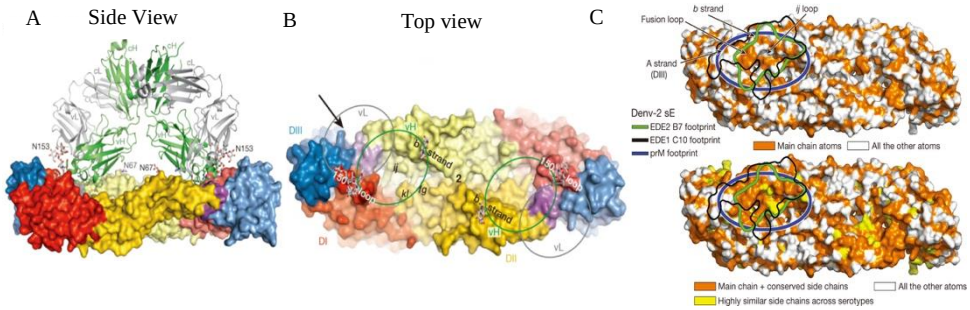


Figure The structures of DENV-2 in complex with anti-EDE-mAb showing the epitope of anti-EDE antibody lies across 2 E within a dimer. A) side view and B) top view. Domain I, II and III of E protein are indicated in red, yellow and blue. On the top view, grey and green ovals show the binding areas of heavy and light chains of the anti-EDE mAb. C) Exposed main-chain atoms in the epitope. Surface representative of DENV-2 sE as viewed from outside the virion with exposed main-chain atoms orange (top) or with main-chain atoms plus conserved side chains in orange, and highly similar side chains in yellow (bottom). The epitopes of two EDE mAbs are indicated.

Training opportunities

The student will join a team having expertise more than 20 years in virology, immunology and molecular biology. Our work has a great contribution in the field and successfully published in high impact journals including Nature, Science, Nature Medicine, Nature Immunology and Nature Communication with high citation. The student will be trained by experienced post-docs in a broad range of techniques such as basic virology (viral propagation, neutralisation and viral titration), immunology (ELISA, Immuno-precipitation, SDS-PAGE, Western blot, FPLC and affinity purification, Flow cytometry, Single cell sorting, tissue/cell culture, molecular biology (PCR, using software programs to design primers, mutagenesis, deep sequencing of antibody repertoire, cloning, protein expression in bacteria, yeast, insect and mammalian cells systems) generating monoclonal antibodies from single human and mouse B cell, using software and structure analysis to design new immunogens, and mouse handling. The project is in collaboration with investigators from Pasteur Institute France, Wellcome Trust Sanger Institute Cambridge UK and Scripps Institute USA. Therefore, apart from meeting with wide range of experts in the campus, the student will have chances to interact/exchange knowledge with their scientists and may have an opportunity to work in their labs.

Supervisor: Professor Gavin Screaton
Co-Supervisor: Dr. Juthathip Mongkolsapaya

Professor Gavin Screaton is a Professor of Medicine, Head of the Medical Science Division, University of Oxford and Consultant Physician. He is a a Fellow of the Academy of Medical Sciences, a Fellow of the Royal College of Physicians, a member of the Association of Physicians. His research, which has been supported by a series of Fellowships awarded by the MRC, Wellcome Trust and European Union FP7 program, has covered a variety of topics from control of RNA processing and apoptosis to immunology. He is a Senior Investigator awarded by Wellcome Trust.

Dr. Juthathip Mongkolsapaya was trained in biochemistry, microbiology and immunology. Her work has been funded by Wellcome Trust, MRC, Newton-MRC and European Union FP7 program. The current interests of our laboratory revolve around the immunology of infectious diseases with a special interest in dengue haemorrhagic fever and ZIKV infection, where our research is currently

funded by the Wellcome Trust and MRC with active research collaborations in UK, South-East Asia, South Americas, and USA.

Key publications

1 Wilder-Smith, A. *et al.* Deliberations of the Strategic Advisory Group of Experts on Immunization on the use of CYD-TDV dengue vaccine. *Lancet Infect Dis*, doi:10.1016/S1473-3099(18)30494-8 (2018).

2 Screaton, G., Mongkolsapaya, J., Yacoub, S. & Roberts, C. New insights into the immunopathology and control of dengue virus infection. *Nat Rev Immunol* **15**, 745-759, doi:10.1038/nri3916 (2015)

3 Dejnirattisai, W. *et al.* A new class of highly potent, broadly neutralizing antibodies isolated from viremic patients infected with dengue virus. *Nat Immunol* **16**, 170-177, doi:10.1038/ni.3058 (2015).

4 Barba-Spaeth, G. *et al.* Structural basis of potent Zika-dengue virus antibody cross-neutralization. *Nature* **536**, 48-53, doi:10.1038/nature18938 (2016).

5 Fernandez, E. *et al.* Human antibodies to the dengue virus E-dimer epitope have therapeutic activity against Zika virus infection. *Nat Immunol* **18**, 1261-1269, doi:10.1038/ni.3849 (2017).

Project 10

Project Title: Structural biology of cell surface receptors

Primary Supervisor: Yvonne Jones - yvonne.jones@strubi.ox.ac.uk

Project Overview
We investigate how one family of extracellular ligands, the semaphorins, work together with their receptors, the plexins, to control the ability of a cell to adhere or to move in a specific direction. Such signals underlie human biology ranging from neural development to immune responses. Similarly, we research the mechanisms by which the Wnt signalling system maintains the exquisite balance between stem cell proliferation and differentiation that is required to avoid neurological and bone disorders as well as cancer. Ultimately, we and others can use this knowledge to inform the design of novel therapeutics, for example our studies on Notum, a modulator of Wnt signalling, which we are currently targeting in structure guided drug design.

Training opportunities
Jones' group members have the opportunity to gain experience of two core structural biology techniques: x-ray crystallography and electron cryo-microscopy. In addition, to achieve our aim of generating insights which span from detailed atomic structure to cellular context we access a broad range of state-of-the-art methodologies as and when required. These include mammalian cell based expression systems, protein purification, biophysical techniques, super-resolution light microscopy and electron cryo tomography. During the pandemic members of the group contributed their expertise to a broad range of the NDM's COVID-19 research activities.

Supervisor
Professor E. Yvonne Jones
Professor Jones holds the Sir Andrew McMichael Professorship of Structural Immunology at the University of Oxford. She is co-Head of the Division of Structural Biology (STRUBI) and Deputy Head of the Nuffield Department of Clinical Medicine (NDM). Within her own research group (funded by the UK Medical Research Council, the ERC and Wellcome) she uses structural and functional analyses to investigate the molecular mechanisms by which cells signal to each other in the human body. Professor Jones' work, built upon strong links with clinically related groups, has provided fundamental insights into signalling systems of importance for cellular immunology, developmental biology and cancer. She has published some 290 research papers and reviews including senior author papers in Nature, Science and Cell. She has been a member of scientific Committees for various funding bodies in the UK and Europe and currently serves on Scientific Advisory Boards for academic Institutes in the UK, France and Switzerland. She is a Fellow of the Royal Society and of the Academy of Medical Sciences as well as a Member of EMBO.

Key publications
1. I.J. McGough[=], L. Vecchia[=], B. Bishop, T. Malinauskas, K. Beckett, D. Joshi, N. O'Reilly, C. Siebold, E.Y. Jones* and J-P. Vincent*. (2020) 'Glypicans shield the Wnt lipid moiety to enable signalling at a distance.' *Nature* **585**, 85-90

2. V. Mehta, K.L. Pang, D. Rozbesky, K. Nather, A. Keen, D. Lachowski, Y. Kong, D. Karia, M. Ameismeier, J. Huang, Y. Fang, A. del Rio Hernandez, J.S. Reader, E.Y. Jones and E. Tzima*. (2020) 'The Guidance Receptor Plexin D1 is a mechanosensor in endothelial cells.' *Nature* **578**, 290-295.

3. A.A. van der Klaauw[=], S. Croizier[=], E. Mendes de Oliveira, L.K.J. Stadler, S. Park, Y. Kong, M.C. Banton, P. Tandon, A.E. Hendricks, J.M. Keogh, S.E. Riley, S. Papadia, E. Henning, R. Bounds, E.G. Bochukova, V. Mistry, S. O'Rahilly, R.B. Simerly, INTERVAL, UK10K consortium, J.E.N. Minchin, I. Barroso, E.Y. Jones, S.G. Bouret* and I.S. Farooqi*. (2019) 'Human Semaphorin 3 variants link melanocortin circuit development and energy balance.' *Cell* **176**, 729-742.

4. Y. Kong[=], B.J.C. Janssen[=], T. Malinauskas, V.R. Vangoor, C.H. Coles, R. Kaufmann, T. Ni, R.J.C. Gilbert, S. Padilla-Parra, R.J. Pasterkamp* and E.Y. Jones* (2016) 'Structural basis for plexin activation and regulation.' *Neuron* **91**, 548-560

5. S. Kakugawa[=], P.F. Langton[=], M. Zebisch[=]*, S. Howell, T.-H. Chang, Y. Liu, T. Feizi, G. Bineva, N. O'Reilly, A.P. Snijders, E.Y. Jones* and J.-P. Vincent*. (2015) 'Notum deacylates Wnts to suppress signalling activity.' *Nature* **519**, 187-192

Project 11

Project Title: Profiling innate immune signalling through the Myddosome and IRAKs using proximity labelling

Primary Supervisor: Andreas Damianou - andreas.damianou@ndm.ox.ac.uk

Project overview

The ability of our body to react to both tissue damage and infection is a highly regulated process which include the formation of supramolecular organizing centres (SMOCs). SMOCs are responsible to link active receptors and signal transduction pathways, which are essential for initiating innate immune responses.

The MYD88 complex (Myddosome), a well-known SMOC, is a post-receptor protein complex, is assembled following signalling through the Toll-like receptors (TLR) and the Interleukin-1 receptor (IL-1R). Upon activation of the Toll-like receptor, the Myddosome complex is assembled including components such as MYD88, IRAK4 and IRAK1/2 kinases. The formation of the Myddosome and subsequently the activation of IRAKs leads to the formation of the TAF6-TAK1-IKK signalosome, which eventually leads to the production of some potent proinflammatory cytokines and chemokines to initiate an acute inflammatory response.

The assembly and disassembly of the Myddosome complex is not yet fully described. Additionally, the Myddosome seems to remain intact in cells even after the induction of acute inflammatory responses. It is not fully established wha thet Myddasome is doing during those late time points. The availability of cutting-edge of mass spectrometry technology as well as molecular tools in the Kessler lab gives us the opportunity to gain unprecedented depth and novel molecular insights into these cellular processes.

Therefore, we are looking for a highly motivated individual with a keen interest in molecular/ cellular biology and experience in programming as well as bioinformatics to i) help in the development of a time course proximitome approach to investigate the assembly of the Myddasome and ii) applying Post-translational modification enrichment methods (Phospho-proteomics, ubiquitomics) in combination of proximity labelling (APEX2) to reveal the essential role of PTMs to regulate those molecular process. iii) We shall apply integrated omics bioinformatics to untangle complex cellular –omics (proteomics / transcriptomics / ubiquitomics) data sets to unravel molecular details of the Myddosome assembly and disassembly dynamics during TLR/IL1 innate immune signalling.

Training opportunities

Introduction to background biology of the cellular innate immune system as well as the cellular ubiquitin system and its function

Training on getting familiar with –omics data, such as transcriptomics, mass spectrometry derived data sets such as proteomics, metabolomics, but also ubiquitomics, interactomics data sets

Introduction to bioinformatics tools to process –omics data, such as R (training courses) and more specialised –omics analysis software including Mascot, MaxQuant, Perseus, SAINT, Progenesis IQ, Proteomics Discoverer, PEAKS, MS Fragger, Fragpipe; possibilities to follow advanced courses on programming (Python, JavaScript, HTML, Elm etc).

Key Publications

1. Pinto-Fernandez A, Salio M, Partridge T, Chen J, Vere G, Greenwood H, Olie CS, Damianou A, Scott HC, Pegg HJ, Chiarenza A, Díaz-Saez L, Smith P, Gonzalez-Lopez C, Patel B, Anderton E, Jones N, Hammonds TR, Huber K, Muschel R, Borrow P, Cerundolo V, Kessler BM. Deletion of the deISGylating enzyme USP18 enhances tumour cell antigenicity and radiosensitivity. Br J Cancer. 2021 Feb;124(4):817-830. doi: 10.1038/s41416-020-01167-y

2. Liang Z, Damianou A, diDaniel E, Kessler BM. Inflammasome activation controlled by the interplay between post-translational modifications: emerging drug target opportunities. Cell Commun Signal. 2021 Feb 24;19(1):23.doi: 10.1186/s12964-020-00688-6.

3. Hung V, Udeshi ND, Lam SS, Loh KH, Cox KJ, Pedram K, Carr SA, Ting AY. Spatially resolved proteomic mapping in living cells with the engineered peroxidase APEX2.
Nat Protoc. 2016 Mar;11(3):456-75. doi: 10.1038/nprot.2016.018

Project 12

Project Title: The interaction between the gut microbiome composition and the host genome

Primary Supervisor: John Todd - jatodd@well.ox.ac.uk

Project Overview

The health and functions of the human gut microbiome are critical for human health, with early development of the interactions between the gut, the child's developing immune system and the commensal bacteria, in the first weeks and months of life being key, and yet still relatively unexplored (https://pubmed.ncbi.nlm.nih.gov/34143954/). Type 1 diabetes (T1D) is a common autoimmune disease which begins in early life where the first signs of autoimmunity against insulin begin around the weaning period. This process is under control of the strongest inherited genetic effects in the human genome for T1D, the immune response genes, HLA class II DR and DQ, that promote the development of tolerance to self-antigens and to commensal bacterial antigens in early life. Class II genes act in the thymus to establish tolerance, ensuing that potentially autoreactive T cells are deleted whilst maintaining a large repertoire of antigen-recognising T cells for immune responses to infection as well as for the generation of self-reactive regulatory T cells. In addition, DR and DQ could be acting to modulate tolerance through the recognition of commensal antigens in the first weeks of life (https://www.nature.com/articles/s41586-021-03531-1?utm_source=feedburner&utm_medium=feed&utm_campaign=Feed%3A+nature%2Frss%2Fcurrent+%28Nature+-+Issue%29).

Our project is to investigate the associations of the highest T1D risk class II genotypes with the commensal microbiome composition versus the most T1D-protective class II genotypes. We are sequencing stool DNA samples from children and investigating these possible genetic associations. Is the early-life microbiome a risk marker for T1D?

Training opportunities

Bioinformatics, genetics, immunology, clinical trials

Supervisor

Professor John Todd

John Todd FRS, FMedSci, FRCP Hons, PhD is Professor of Precision Medicine at the University of Oxford, Director of the Wellcome Centre for Human Genetics and of the JDRF/Wellcome Diabetes and Inflammation Laboratory (DIL), and an Emeritus Senior Investigator of the National Institute for Health Research. Previously, Todd was Professor of Human Genetics and a Wellcome Trust Principal Research Fellow at the University of Oxford, and until 2016, Professor of Medical Genetics at the University of Cambridge. His PhD was in Biochemistry at the University of Cambridge, followed by a postdoctoral fellowship at Stanford University. Todd researches type 1 diabetes (T1D) genetics and disease mechanisms with the goal of delivering clinical interventions. Todd helped pioneer genome-wide genetic studies in common diseases. He then went on to study the associations between disease-associated genetic variants and phenotypes in T1D by founding and deploying the Cambridge BioResource. In the latest phase of his research, to translate basic genetic and immunological knowledge to treatment and prevention, the DIL is testing the efficacy of ultra-low doses of interleukin-2 in newly-diagnosed children with T1D to preserve the remaining pancreatic islet beta-cell insulin production and investigating the role of the microbiome in T1D. Todd is also

part of the international consortium, Global Platform for the Prevention of Autoimmune Diabetes (GPPAD), aiming to establish primary preventions of T1D in randomised placebo-controlled trials, initially by testing the possibility that daily oral insulin given to children at high genetic risk of T1D can inhibit the autoimmunity that causes T1D. His research in genetics and diabetes has received several awards and prizes, including the 1995 Minkowski Prize of the European Association for the Study of Diabetes (EASD) and the 2021 EASD/Novo Nordisk Foundation Diabetes Prize for Excellence. Todd has supervised 40 PhD and MSc students with three in progress and has an h-index of 131 and over 40,000 citations.

Key publication

Peripheral tolerance to insulin is encoded by mimicry in the microbiome
Arcadio Rubio García, Athina Paterou, Mercede Lee, Hubert Sławiński, Linda S. Wicker, John A. Todd, Marcin Ł. Pękalski
bioRxiv 2019.12.18.881433; doi: https://doi.org/10.1101/2019.12.18.881433

Project 13

Project Title: Elucidating the body-brain axis using multi-organ population imaging

Primary Supervisor: Anya Topiwala - anya.topiwala@bdi.ox.ac.uk
Additional Supervisor: Thomas Nichols - thomas.nichols@bdi.ox.ac.uk

Project Overview
The specific project subject can be tailored to the interests of the student.
Physical and mental health are intrinsically linked, but the exact pathways between them are poorly understood. For the first time, large population samples with multi-organ imaging resources offer an opportunity to provide clarity.

The aim of this project is to elucidate how heart and liver health link with brain health in determining later mental health.

The project will entail learning state-of-the-art neuroimaging and epidemiological analysis techniques to achieve these goals.

Training opportunities
Training opportunities include: epidemiology, imaging analysis, statistical methods and research dissemination through supervision and formal courses, including the renowned FMRIB graduate program.

Supervisors
Anya Topiwala
Dr Topiwala studied clinical medicine at University of Oxford. Subsequently she pursued psychiatry training, and was awarded MRCPsych in 2009. She was a Clinical Lecturer in Older Adult Psychiatry at the University of Oxford between 2012-7. Alongside her clinical training she completed a DPhil in Psychiatry at the interface of neuroimaging and epidemiology. In 2019 she was awarded a Wellcome Trust Clinical Development Fellowship to study impact of the alcohol consumption on brain health. For this work she is based at the Big Data Institute, but collaborates with academics at Oxford, UCL, LSHTM, Cambridge, Yale and Harvard universities.
She qualified as a Consultant Psychiatrist in 2017, and worked in OUH until the award of her research fellowship. She now holds an Honorary Consultant contract at Oxford Health Foundation Trust where she runs a weekly memory clinic. She also holds a Postgraduate Diploma in Higher Education and Teaching (2017).

Thomas Nichols
Professor Nichols is a Professor of Neuroimaging Statistics and a Wellcome Trust Senior Research Fellow. His background includes industrial and academic experience. He served on the faculty of the University of Michigan's Department of Biostatistics and subsequently became the Director of Modelling and Genetics at GlaxoSmithKline's Clinical Imaging Centre, London. He was based at the University of Warwick from 2009 to 2017 when he moved to the BDI in Oxford to lead the statistical neuroimaging group. His extensive experience includes modelling large, complex data. He has gained particular notoriety for multiple testing inference for brain imaging and methods for integrating genetic and imaging data.

Key publications

1. **Topiwala A**, Taschler B, Ebmeier KP, Smith S, Zhou H, Levey DF, Codd V, Samani NJ, Gelernter J, **Nichols TE**, Burgess S. Alcohol consumption and telomere length: observational and Mendelian randomization approaches. *medRxiv* 2021.

2. **Topiwala A**, Ebmeier KP, Maullin-Sapey T, **Nichols TE**. No safe level of alcohol consumption for brain health: observational cohort study of 25,378 UK Biobank participants. *medRxiv* 2021

3. **Topiwala A,** Allan C, Valkanova V, Zsoldos E, Filippini N, Sexton C, Mahmood A, Fooks P, Singh-Manoux A, Mackay C, Kivimaki M, Ebmeier KP. Moderate alcohol consumption as a risk factor for adverse brain outcomes at older ages: a 30-year prospective cohort study. *BMJ 2017 357:j2353.*

4. Smith SM, **Nichols TE**. Statistical challenges in "big data" human neuroimaging. *Neuron* 2018. 97(2): 263:8.

5. Winkler AM, Ridgway GR, Webster MA, Smith SM, **Nichols TE**. Permutation inference for the general linear model. *Neuroimage* 2014. 92: 381-97.

Project 14

Project Title: A multi-omic approach to investigating the host response to severe infection

Primary Supervisor: Julian Knight - julian@well.ox.ac.uk

Project Overview
This proposal aims to provide training in genomic medicine with particular application to immunology and infectious disease, combining a high-quality scientific research project focused on investigating the basis of a dysregulated response to severe infection with clinical experience in genomic medicine and internal medicine in the Oxford University Hospitals NHS Trust. The research will be collaborative, based in Oxford at the laboratory of Professor Julian Knight at the Wellcome Centre for Human Genetics while working closely with colleagues in Oxford and in China as part of CAMS-Oxford Institute.

Research proposal
Managing patients with severe infection remains a major clinical challenge. Here, dysregulation of the normally appropriate host immune response is important to pathogenesis. This occurs only in a small minority of patients with infections but represents a major burden of disease as highlighted by the current COVID-19 pandemic and conditions such as sepsis which is the most common reason for admission to medical intensive care units (ICUs). There are currently few effective treatments for these patients, for example sepsis has a persistently high mortality of 25-30% despite optimal available therapy. COVID-19 illustrates the urgent need to understand why severe disease develops in some patients and how knowledge of disease pathogenesis may enable improved treatments.

Inherited factors are important, both specifically for sepsis and COVID-19 together with other instances where severe infections are seen with both highly penetrant rare mutations (classically resulting in primary immunodeficiency disorders) and more common genetic variants. Knowledge of such experiments of nature provides new insights into function of the immune system and how it becomes dysfunctional in disease.

This research project will address the question of how and why some patients develop severe infections focusing on sepsis, COVID-19 and related infections. This will involve combining analysis of genetics with multi-omic studies to understand the nature and basis of variation in immune function and clinical outcome in response to infection.

The work will build on ongoing research in the Knight lab. We have established a major bioresource of patients admitted to ICUs with sepsis in the UK through the Genomic Advances in Sepsis (GAinS) study. This has enabled identification of genetic markers associated with reduced mortality in sepsis. For example, variants in *FER* (regulating leukocyte recruitment in response to lipopolysaccharide (LPS)) were found through the first genome-wide association study (GWAS) of sepsis survival.

We have discovered that distinct patterns of leukocyte gene expression occur in adult sepsis patients (sepsis response signatures, SRS). These define specific novel disease endotypes that are informative for the underlying immune response state and outcome, that are robust to source of infection and have been independently validated. We further find that membership of disease

endotypes cannot be established from clinical covariates and in a subset of patients are dynamic over time. Importantly, these sepsis endotypes predict response to therapy.

We determined that genetic differences can modulate the individual transcriptomic response to sepsis, and to bacterial endotoxin in healthy volunteers, through key immune and metabolic response genes and networks, including the hypoxic response and the switch to glycolysis, endotoxin tolerance and T cell exhaustion. We have also identified genetic variants associated with invasive bacterial disease. For example, by whole exome sequencing patients with group A streptococcal necrotising fasciitis we have identified rare deleterious variants in genes involved in tissue structure and epithelial integrity.

We have an ongoing major programme of work through the COVID-19 Multi-omic Blood ATlas (COMBAT) Consortium to understand the immune signatures and drivers of variation in the response to COVID-19. This includes single cell transcriptomics, proteomics and epigenomics with flow and mass cytometry, plasma proteomics using mass spectrometry and immunoassays, and whole blood total RNA profiling to identify hallmarks of disease severity together with shared and specific features on comparison with pre-pandemic all cause sepsis and influenza.

Training Opportunities
The clinician undertaking this project will gain a comprehensive research training in genomics together with related expertise in immunology and infectious disease. This will include training in bioinformatics and statistical genetics as well as functional genomics. The student will benefit from relevant modular teaching available through the Genomic Medicine and Statistics DPhil programme (for which Professor Knight is the Course Director) and the Medical Sciences Doctoral Training Centre. Clinical training in internal medicine will be enabled through attachment to a clinical firm at the John Radcliffe Hospital under the supervision of Professor Knight (Honorary Consultant) for one month per year. The student will also benefit from involvement in translational genomics research through the NHS Genomic Medicine Service Alliance (Central and South region) (where Professor Knight is Research Director).

Supervisor
Professor Julian Knight
Professor Knight is Professor of Genomic Medicine at the University of Oxford, Honorary Consultant Physician in Internal Medicine at the Oxford University Hospitals NHS Trust, and a Fellow and Tutor in Medicine at Merton College. His research investigates how genetic variation between individuals modulates genes critical to mounting an appropriate immune and inflammatory response and may contribute to susceptibility to autoimmune and infectious disease (https://www.well.ox.ac.uk/research/research-groups/julian-knight-group).

Key publications
1. Kwok AJ, Mentzer A & Knight JC. 2021 Host genetics and infectious disease: new tools, insights and translational opportunities. *Nat Rev Genet* **22**, 137-153.
COMBAT Consortium. 2021 A blood atlas of COVID-19 defines hallmarks of disease severity and specificity. medRxiv, 2021.05.11.21256877.

2. Antcliffe DB, Burnham KL, Al-Beidh F, Santhakumaran S, Brett SJ, Hinds CJ, Ashby D, Knight JC, Gordon AC. 2018 Transcriptomic Signatures in Sepsis and a Differential Response to Steroids: From

the VANISH Randomized Trial. *American Journal of Respiratory and Critical Care Medicine* **199**, 980-986

3. Burnham KL, Davenport EE, Radhakrishnan J, Humburg P, Gordon AC, Hutton P, Svoren-Jabalera E, Garrard C, Hill AVS, Hinds CJ & Knight JC. 2017 Shared and Distinct Aspects of the Sepsis Transcriptomic Response to Fecal Peritonitis and Pneumonia. *Am J Respir Crit Care Med* **196**: 328-339.

4. Davenport EE, Burnham KL, Radhakrishnan J, Humburg P, Hutton P, Mills TC, Rautanen A, Gordon AC, Garrard C, Hill AV, Hinds CJ & Knight JC. 2016 Genomic landscape of the individual host response and outcomes in sepsis: a prospective cohort study. *Lancet Respir Med* **4**: 259-71.
Fairfax BP, Humburg P, Makino S, Naranbhai V, Wong D, Lau E, Jostins L, Plant K, Andrews R, McGee C & Knight JC. 2014 Innate immune activity conditions the effect of regulatory variants upon monocyte gene expression. *Science* **343**: 1246949.

5. van der Poll T, van de Veerdonk FL, Scicluna BP & Netea MG. 2017 The immunopathology of sepsis and potential therapeutic targets. *Nat Rev Immunol* **17**: 407-420.

Project 15

Project Title: Integrative genomic approaches to evaluate the interaction between the tumour microenvironment, immunity and obesity

Primary Supervisor: Francesca Buffa - francesca.buffa@oncology.ox.ac.uk
Additional Supervisors: Skirmantas Kriaucionis - skirmantas.kriaucionis@ludwig.ox.ac.uk; Simon Lord - simon.lord@oncology.ox.ac.uk

Project Overview:
The tumour microenvironment plays a key role in cancer progression and in mediating response to many therapies. It is expected that within the next decade obesity will overtake smoking as the primary environmental cause of cancer in the Western World. Growing evidence suggests that tumour infiltrating immune cell function in breast cancer may be altered in obesity but this relationship is poorly understood. Numerous experimental and clinical studies have established the importance of inflammation and immunity in the development of obesity and its metabolic complications, including insulin resistance and type 2 diabetes mellitus. Emerging patient data points toward obesity dependent differences in immune cell behaviour playing a key role in defining breast cancer response to chemotherapy. For example, pathological complete response (pCR) to neoadjuvant chemotherapy is a strong predictive marker of good outcome in triple negative breast cancer. However, a pooled analysis of patients recruited to 13 prospective clinical trials showed that obese breast cancer patients had lower pCR rates compared to those of normal weight (Wang et al, Cancer Res, 2020). Moreover, in another study of 450 breast cancer patients, high levels of stromal infiltrating lymphocytes predicted pCR and favourable prognosis in lean patients but not obese patients (Floris et al, JNCI, 2020).

Published pre-clinical data also links altered immune cell phenotype in obesity with tumour behaviour. For example, obese mouse models of breast cancer developed lung neutrophilia which linked to increased breast metastasis at this site and transcriptomic analysis revealed gene expression changes associated with a pro-metastatic phenotype (Quail et al, Nat Cell Biol, 2017). Elevated plasma cholesterol in mice fed a high fat diet has been shown to alter myeloid immune cell function resulting in a prometastatic effect (Baek et al, Nat Comm, 2017). Growing preclinical evidence suggests that the transcription factor STAT3 is a key driver of the effects of obesity on immune cell function in the context of cancer. STAT3, a major downstream mediator of leptin signalling, is commonly overexpressed in breast cancer and associated with progression, metastasis and immune evasion. Wang et al showed, using obese mouse models, that leptin stimulated induction of STAT3 increased PD-1 expression and memory T-cell exhaustion (Wang et al, Nat Med, 2019). Another group showed that leptin induced STAT3 signalling led to increased fatty acid oxidation in CD8+ T cells in breast cancer models blunting immune response. Inhibition of fatty acid oxidation led to increased CD8+ T cell glycolysis and anti-tumour function (Zhang et al, Cell Metab, 2018).

Bulk RNA sequencing can identify transcriptomic changes between tumours that have arisen in obese and lean patients. For example, analysis of microarray and RNASeq data from a clinical trial and the TGCA database identified greater expression of angiogenic pathways and reduced immune checkpoint gene expression for clear cell renal cancers that had arisen in obese patients (Sanchez et al, Lancet Oncol, 2020). However, human tumour samples including breast tumours contain many

different cell types besides epithelial cancer cells including immune cells, adipocytes, stromal cells and endothelial cells. Whilst deconvolution techniques of bulk sequencing data provide some insight into different cellular compartments, single cell sequencing provides far higher phenotypic resolution.

In this project we plan to carry out single cell sequencing of immune cells from breast tumours harvested from lean and obese patients using a microfluidic assay that is already optimised by our team alongside detailed patient anthropometric and metabolic measurements of obesity. We shall also profile the transcriptomic signature of other cell types within the tumour microenvironment such that we can interrogate crosstalk between these different tumour compartments. 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, including adipocytes, fibroblasts and endothelial cells. We shall develop capability to carry out both transcriptomic and epigenetic characterisation of several tumour compartments and develop mathematical modelling and bioinformatic techniques to identify links between the microenvironment and a) tumour and b) immune cell phenotype. The project will draw on cross-departmental expertise: Simon Lord (breast cancer clinician, drug development, tumour metabolism and tissue collection), Francesca Buffa (bioinformatics, tumour metabolism), Skirmantas Kriaucionis (Ludwig Institute, single cell DNA methylation sequencing), Kim Midwood (Kennedy Institute, cellular environment/immune cell interaction, single cell sequencing), and Adrian Harris (tumour metabolism, drug development).

Ethical approval, single cell sequencing protocols and a pipeline for tissue collection alongside clinical measurements is already established alongside some matched funding. All patients going on study will have their anthropometric measurements recorded and undergo bioimpedance measurements and a dual energy Xray absorbtiometry (DEXA scan) to measure body composition at an onsite dedicated facility based in the Oxford Centre for Diabetes, Endocrinology and Metabolism (OCDEM). Tomiyama et al showed that 30% of individuals classified as being healthy based on their BMI scores were in fact metabolically unhealthy on clinical evaluation. Additionally, a fasting blood sampling will be undertaken to measure serum glucose, insulin, HbA1c and leptin. Tumour samples will be freshly collected via biopsy of consented patients during breast surgery (ongoing collection of samples in this fashion already established).

We shall use the 10X genomics single cell RNAseq platform in the Ludwig Institute for Cancer Research at the University of Oxford. This method has already been optimised in the Kriaucionis lab. Supervision of the bioinformatic analysis will be led by Professors Kriaucionis and Buffa. A focus will be placed on the phenotypic analysis of transcriptomic data derived from tumour cells, T cells and macrophages. The Kriaucionis group have already optimised this technique for human breast cancer samples (see **Figure 1**) and have developed techniques that facilitate efficient preservation of adipocytes during dissociation of biopsies, a particular challenge. 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.

We have already obtained ethical approval (REC 19/SC/0025) for the collection of fresh breast tumour samples (biopsied directly on the surgical table) from obese and lean patients and have ongoing collection of samples with matched clinical data including body composition DEXA scans and blood sampling for measures of host patient metabolism. Funding for collection and substantial bioinformatic analytical support is already in place (see below).

Initial research objectives

Primary research objective:

1. Map the differential expression of markers of T-cell exhaustion and macrophage polarity between tumours in lean and obese patients

Exploratory research objectives:

1. Map the differential expression of glycolysis, fatty acid oxidation, oxidative phosphorylation and nucleotide metabolism pathways across different tumour compartment cell types in lean and obese patients
2. Evaluate differences in expression of STAT3 regulated genes and genes that regulate the fatty acid oxidation and glycolysis pathways between obese and lean T-cell populations
3. Pilot work to optimise preparation of samples and analysis for single cell DNA methylation sequencing

Line of sight:

1. Match novel assays to single cell sequencing to understand crosstalk between different compartments of the tumour microenvironment in context of obesity
    - Carbon tracing in primary culture organoid models
    - Modelling of tumour matrix and interaction with T-cell/macrophage compartment
    - Validation using spatial transcriptomic analysis of tissue sections
    - Sampling of normal tissue from ipsilateral breast (ethical approval obtained) to compare paired normal
2. Catalyst for drug development projects in partnership with the Oxford Centre for Medicines Discovery

Training opportunities:

The student will be mentored via weekly one-to-one meeting with the supervisors. They will regularly present their results at the Kriaucionis and Buffa lab meetings, and will benefit from a range of cutting-edge bioinformatics and laboratory tools and training. They will have the opportunity to gain skills in basic laboratory skills, primary tissue culture, interpretation of mass spectrometry data, and single cell sequencing. Additionally, the student will have the opportunity to develop clinical study protocols and gain experience of the required regulatory 'hurdles' under supervision of Dr Lord.

Supervisors:

<u>Professor Francesca Buffa</u>

Francesca Buffa is Professor of Computational Biology and Cancer Genomics in the Department of Oncology at the University of Oxford. She leads a systems biology laboratory funded by the European Research Council and Cancer Research UK. We develop and apply advanced

computational, machine learning and mathematical modelling approaches to the analysis of cutting-edge multi-omics and functional data, produced in our laboratory and collaborating laboratories, to understand cancer as a complex system.
Our main projects include:

- Gene network reconstruction in bulk samples and single cells to study transcriptional regulation, cell signalling and cell-cell communication
- Machine learning in large clinical cohorts and biobanks to derive integrated multi-omics classifiers

Development of omics 'signatures' to infer functional status, phenotype and clinical outcome of human samples

Professor Skirmantas Kriaucionis

Skirmantas Kriaucionis is Associate Professor and leads the Epigenetic Mechanisms Group at the Ludwig Institute for Cancer Research at the University of Oxford. His research programme aims to elucidate the molecular function of DNA modifications in normal cells and cancer by employing biochemistry and in vivo approaches to investigate roles of DNA modifications in transcription, heritability, mutability and nuclear organisation.

Clinical supervisor:

Dr Simon Lord

Simon Lord has been Senior Clinical Researcher in Experimental Cancer Therapeutics in the Department of Oncology since 2015 and is a consultant medical oncologist at the Oxford Cancer Centre. In 2021 he was appointed Director of the Early Phase Clinical Trials Unit in the Department of Oncology at the University of Oxford. His research interests focus on the development of new drugs to treat breast cancer and he also leads a number of clinical studies assessing how novel metabolic imaging signatures may reflect tumour biology.

Key publications:

1. **Lord S**, Cheng W, Liu D, Gaude E, Haider S, Metcalf T, Patel N, Teoh EJ, Gleeson, F, Bradley K, Wigfield S, Zois C, McGowan D, Ah-See M, Thompson A, Sharma A, Bidaut L, Pollak M, Roy PG, Karpe F, James T, English R, Adams R, Campo L, Ayers L, Snell C, Roxanis I, Frezza C, Fenwick JD, **Buffa FM**, **Harris AL**. Integrated pharmacodynamic analysis identifies two metabolic adaption pathways to metformin in breast cancer. Cell Metabolism. 2018 Nov;28(5):679-688.

2. Zauri M, Berridge G, Thezenas M, Pugh K, Goldin R, Kessler BM, **Kriaucionis S**. CDA directs metabolism of epigenetic nucleosides revealing a therapeutic window in cancer. Nature. 2015 Aug;524(7563):114-118.

3. Endonuclease enrichment TAPS for cost-effective genome-wide base-resolution DNA methylation detection. Cheng J, Siejka-Zielinska P, Liu Y, Anandhakumar C, **Kriaucionis S**, Song C. Nucleic Acids Res. 2021 Jul 21;49(13):e76

4. Modeling genotypes in their microenvironment to predict single- and multi-cellular behaviour. Voukantsis D, Kahn K, Hadley M, Wilson R, **Buffa FM**. GigaScience. 2019 Mar;8(3):1-15

5. Matrix-targeting immunotherapy controls tumor growth and spread by switching macrophage phenotype. Deligne C, Devadarssen M, Gammage AN, Gschwandtner M, Erne W, Loustau T,

Marzeda AM, Carapito R, Paul N, Velazquez-Quesada I, Mazzier I, Sun Z, Orend G, **Midwood KS**. Cancer Immunol Res. 2020 Mar;8(3)368-382

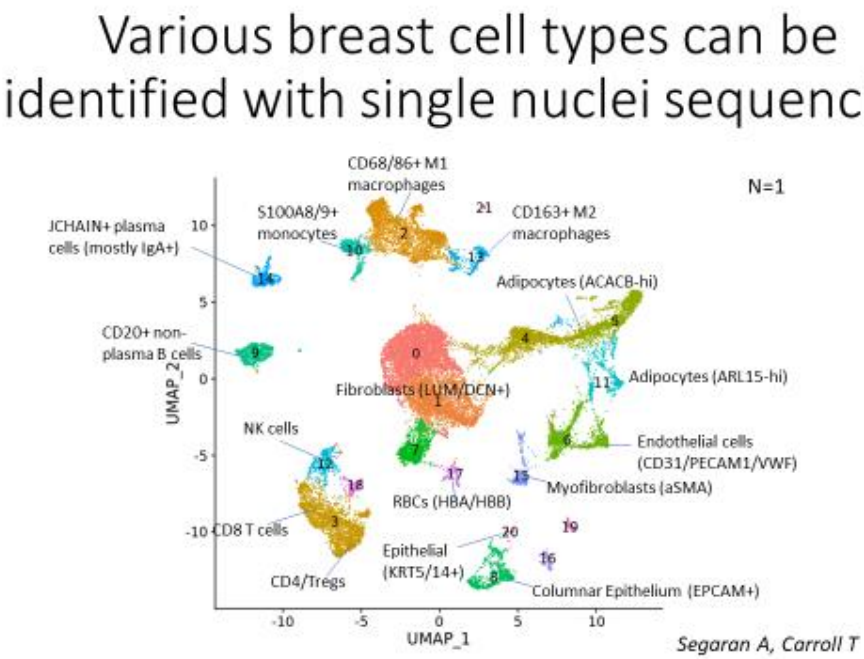


Figure 1: Example of sequencing map from a breast cancer sample (final protocol following optimisation)

Project 16

Project Title: Investigating the molecular and cellular basis of antibody affinity maturation in germinal centres

Primary Supervisor: Oliver Bannard - oliver.bannard@ndm.ox.ac.uk

Project overview

Antibodies are essential components of adaptive immunity and their induction is critical for the effectiveness of almost all vaccines. The efficacy of vaccines depends on various critical parameters including; that antibodies recognize specific (e.g., neutralizing) epitopes on pathogens, that the affinity of the antibody binding is sufficiently strong, that memory and plasma cells (the effectors of humoral immunity) are made in sufficient numbers, and that the induced memory/plasma cells are sufficiently long lived. Our lab is interested in understanding the biology responsible for determining these things – all of which are programmed in germinal centres. These issues are hugely important because efforts to develop effective vaccines against various important pathogens such as HIV and malaria have so far failed.

The quality and affinity of antibodies improves over the course of immune responses through a remarkable process known as affinity maturation, which occurs in germinal centres. Here, germinal centre B cells engage in a greatly accelerated form of Darwinian evolution that involves them deliberately introducing random somatic point mutations into their immunoglobulin variable region (antibody encoding) genes at a rate of approximately 1-2 nucleotide changes per cell, per day (a million times higher than background rates). Newly mutated B cells then compete with each other, based upon their ability to gather antigen, for survival inputs and for cues that drive their further clonal expansion. As such, “better” clones are preferentially “selected”. Affinity improvements are the product of iterative rounds of this mutation and selection process. However, the molecular and cellular events involved in selection are only partially understood (discussed in detail in Bannard and Cyster, Current Opinions Immunology 2017, PMID 28088708). Some GC B cells then differentiate into memory and plasma cells, however what determines if and when this will occur is not known.

The Bannard lab seeks to develop and apply novel genetic *in vivo* tools to investigate these issues. DPhil opportunities exist in the lab to investigate a range of fundamental questions in germinal centre biology. Our current interests include:

- To understand the cellular and molecular events/mechanisms involved in the selection of germinal centre B cells.
- To investigate the cues and molecular events that lead to GCs to generate memory and plasma cells.
- To determine how antibody responses focus on, and develop towards, particularly important but potentially challenging epitopes during viral infections.
- To understand how GC responses might be manipulated to give qualitatively and quantitatively better responses after vaccination.

Projects may involve the development and use of sophisticated genetic modified host/viral models. There will be opportunities to learn and implement cutting edge imaging, sequencing, single B cell

cloning and flow cytometry technologies. As such, students can expect to receive sound intellectual and practical science training.

Informal enquiries are welcomed and can be directed to oliver.bannard@ndm.ox.ac.uk.

Training opportunity

The projects will suit someone with a strong interest in mechanistic aspects of immune responses and who enjoys thinking deeply about complex problems. The student will learn to conduct *in vivo* experiments in mice. Approaches are likely to include single cell genomics, next-generation sequencing, transgenic mouse generation and experimentation, bone marrow reconstitution (including retrovirally transduced bone marrow), confocal microscopy, live cell imaging and multi-color flow cytometry. There will also be opportunities to develop and manipulate viruses. Other immunology, imaging and molecular biology techniques will be employed as needed. This study will be supervised by Dr. Oliver Bannard and will be conducted in the Weatherall Institute of Molecular Medicine (WIMM) as part of the MRC Human Immunology Unit.

Students will be enrolled on the MRC WIMM DPhil Course, which takes place in the autumn of their first year. Running over several days, this course helps students to develop basic research and presentation skills, as well as introducing them to a wide-range of scientific techniques and principles, ensuring that students have the opportunity to build a broad-based understanding of differing research methodologies.

Generic skills training is offered through the Medical Sciences Division's Skills Training Programme. This programme offers a comprehensive range of courses covering many important areas of researcher development: knowledge and intellectual abilities, personal effectiveness, research governance and organisation, and engagement, influence and impact. Students are actively encouraged to take advantage of the training opportunities available to them.

As well as the specific training detailed above, students will have access to a wide-range of seminars and training opportunities through the many research institutes and centres based in Oxford.

Supervisor

Dr Bannard is currently Associate Professor and Wellcome Trust Senior Research Fellow in the Nuffield Department of Medicine and an affiliate member of the MRC Human Immunology Unit. Dr Bannard’s research focusses on adaptive immunity; particularly antibody mediated immune responses, with a special interest in germinal centres.

Key publications

1. Radtke D., Bannard O., 2019. Expression of the Plasma Cell Transcriptional Regulator by Dark Zone Germinal Centre B Cells During Periods of Proliferation. Front. Immunol. 9; 9:3106

2. Stewart, I., Radtke, D., Phillips, B., McGowan, S.J., Bannard, O., 2018. Germinal Center B Cells Replace Their Antigen Receptors in Dark Zones and Fail Light Zone Entry when Immunoglobulin Gene Mutations are Damaging. Immunity 49, 477–489.e7. doi:10.1016/j.immuni.2018.08.025

3. Bannard, O., Cyster, J.G., 2017. Germinal centers: programmed for affinity maturation and antibody diversification. Curr Opin Immunol 45, 21–30. doi:10.1016/j.coi.2016.12.004

4. Bannard, O., McGowan, S.J., Ersching, J., Ishido, S., Victora, G.D., Shin, J.-S., Cyster, J.G., 2016. Ubiquitin-mediated fluctuations in MHC class II facilitate efficient germinal center B cell responses. J Exp Med 213, 993–1009. doi:10.1084/jem.20151682

5. Bannard, O., Horton, R.M., Allen, C.D.C., An, J., Nagasawa, T., Cyster, J.G., 2013. Germinal center centroblasts transition to a centrocyte phenotype according to a timed program and depend on the dark zone for effective selection. Immunity 39, 912–924. doi:10.1016/j.immuni.2013.08.038

Project 17

Project Title: Origins of Genotoxic metabolism and the DNA damage response in stem and cancer cells

Primary Supervisor: KJ Patel - ketan.patel@imm.ox.ac.uk

Project Overview
Our work has shown that metabolism both generalized and intrinsic to blood stem cells unleashes reactive metabolites such as the aldehydes – formaldehyde and acetaldehyde. Such metabolites damage DNA causing the stem cells to die or to accumulate cancer causing mutations. Fortunately, a two-tier protection mechanism ensures that these aldehydes do not irreversibly damage these stem cells. Tier 1 protection consist of enzymes that remove such metabolites whilst tier 2 consists of DNA repair mechanisms the fix metabolite inflicted DNA damage. The aim of the research of the research projects are:

1. To identify by way of genome wide CRISPER –Cas genetic screens in primary stem cells and cell lines new tier 1 protection enzymes and their interaction with specific DNA repair pathways.
2. To identify new genotoxic metabolites that the new tier 1 enzymes remove.
3. To define approaches by which inhibition of two-tier protection might be exploitable to kill certain cancer cells.

These three broad questions will be addressed by using state of the art genetic and system biological approaches. It is anticipated that the answers to these questions may have important implications for stem cell biology, the ageing process and the origin of certain cancers. Interested applicants are encouraged to contact KJ Patel for further inquiries (ketan.patel@imm.ox.ac.uk)
Links:
https://www.rdm.ox.ac.uk/people/kj-patel

Training opportunities
the candidate will gain extensive and in-depth exposure and training in advanced molecular biological and genome engineering technology. The Patel lab takes a comprehensive approach to addressing challenging scientific questions – this ranges from biochemical approaches and cell free reconstitution (Hodskinson et al Nature 2020), next generation whole genome sequencing approaches (Garaycochea et al Nature 2018), chemical biological approaches (Barragan et al Nature 2017), to the creation of transgenic animal models.

Key Publications
1. Garaycoechea, J.I., Crossan, G.P., Langevin, F., Mulderrig, L., Louzada, S., Yang, F., Guilbaud, G., Park, N., Roerink, S., Nik-Zainal, S., Stratton, M.R., Patel, K.J. (2018)
Alcohol and endogenous aldehydes damage chromosomes and mutate stem cells. *Nature* 553: 171-177

2. Burgos-Barragan, G., Wit, N., Meiser, J., Dingler, F.A., Pietzke, M., Mulderrig, L., Pontel, L.B., Rosado, I.V., Brewer, T.F., Cordell, R.L., Monks, P.S., Chang, C.J., Vazquez, A., Patel, K.J (2017)

Mammals divert endogenous genotoxic formaldehyde into one-carbon metabolism. *Nature* 548: 549-554

3. Pontel, L.B., Rosado, I.V., Burgos-Barragan, G., Garaycoechea, J.I., Yu, R., Arends, M.J., Chandrasekaran, G., Broecker, V., Wei, W., Liu, L., Swenberg, J.A., Crossan, G.P., Patel, K.J. (2015) Endogenous formaldehyde is a hematopoietic stem cell genotoxin and metabolic carcinogen. *Molecular Cell* 60: 177-188

4. Hodskinson MH, Bolner A, Sato K, Kamimae-Lanning A.N,Rooijers K, Witte M, Mahesh M, Silhan J, Petek M, Williams DW, Kind J, Chin J, Patel KJ*, Knipscheer P*. (2020) 'Alcohol derived inter-strand crosslinks are repaired by two distinct mechanisms'. Nature 579:603-608

Project 18

Project Title: Iron and the anti-tumour immune response

Primary Supervisor: Hal Drakesmith - alexander.drakesmith@ndm.ox.ac.uk

Project Overview
Activating host T-cell responses against tumours can significantly improve the outcome of a number of cancers. To be effective, these antigen-specific lymphocyte responses have to be large, systemic, functional, and long-lived. Understanding the factors that can limit anti-tumour immunity is important to guide therapy and to design better protocols for improving patient responses. Children with a rare mutation impairing cellular iron uptake have profoundly impaired immunity (Jabara et al). In mice we have established that the size and efficacy of immune responses to a range of vaccines and to influenza virus infection is controlled by the amount of iron that is available to the responding lymphocytes (Frost et al), because iron is critical for T-cell metabolism (Teh et al). In humans with melanoma undergoing checkpoint inhibitor therapy, we found that checkpoint inhibitor therapy led to transcriptional changes in responding CD8+ T cells that enable efficient iron acquisition (Fairfax et al). However, iron deficiency and anaemia are common in cancer patients (Busti et al), and so iron availability to lymphocytes is likely to be frequently suboptimal at a time when the anti-tumour immune response needs iron to develop. We propose a study to establish whether the immune responses to tumours, induced either by vaccination or by checkpoint inhibitor therapy or by CAR-T cells, can be regulated by iron deficiency and iron acquisition. Some of this work will be in mice, but there will be parallel work with human cells and samples from patients to assess how iron regulates T-cells in the context of cancer.

Training opportunities
The project will be based at the MRC Human Immunology Unit in the MRC Weatherall Institute of Molecular Medicine. Facilities available at this location can be found on the Institute's website. For this specific project the training will involve animal work (vaccination, tumour models, manipulation of iron status), in vitro cell culture, flow and mass cytometry, histology and tissue imaging, and bioinformatic analysis of high dimensional datasets. Training and facilities for all these skillsets is available in the institute.

Supervisor
Hal Drakesmith is Professor of Iron Biology and has a lab in the MRC Human Immunology Unit. I have supervised 8 PhD students and have longstanding expertise at the interface of iron, immunity, haematology and infection. Recent work in the lab has been focussing on the effect of iron and hepcidin on adaptive immune responses to infections and vaccinations, and this project on tumour immunity will therefore be a natural extension of the concepts we have established. We have all the tumour mouse models functioning. https://www.imm.ox.ac.uk/research/units-and-centres/mrc-human-immunology-unit/research-groups/drakesmith-group-iron-and-immunity

Clinical supervisors
Ben Fairfax (https://www.imm.ox.ac.uk/people/benjamin-fairfax)
We will also receive advice from Paul Klenerman (https://www.ndm.ox.ac.uk/team/paul-klenerman)

Key publications

1. Busti F et al. Anemia and Iron Deficiency in Cancer Patients. Pharmaceuticals. 2018 Sep 30;11(4)

2. Fairfax BP et al, Peripheral CD8 $^{+}$ T cell characteristics associated with durable responses to immune checkpoint blockade in patients with metastatic melanoma. Nat Med 2020 Feb;26(2):193-199

3. Frost JN et al, Hepcidin-mediated hypoferremia disrupts immune responses to vaccination and infection. Med 2021 Feb 12;2(2):164-179.e12. DOI: 10.1016/j.medj.2020.10.004

4. Jabara HH et al, A missense mutation in TFRC, encoding transferrin receptor 1, causes combined immunodeficiency. Nat Genet. 2016 Jan;48(1):74-8. doi: 10.1038/ng.3465.

5. Teh M et al, Analysis of Iron and Iron-Interacting Protein Dynamics During T-Cell Activation. Front. Immunol. 12 August 2021. https://doi.org/10.3389/fimmu.2021.714

Project 19

Project Title: Hypoxia and HLA-E antigen presentation

Primary Supervisor: Andrew McMichael - andrew.mcmichael@ndm.ox.ac.uk
Additional Supervisor: Jane McKeating - jane.mckeating@ndm.ox.ac.uk

Project Overview:
HLA-E is a non-polymorphic major histocompatibility complex (MHC) class I molecule that is important in the regulation of innate immunity. Its major function is to bind a single peptide, derived from classical MHC class signal sequence, and to then bind the NKG2A receptor on natural killer (NK) cells and thereby inhibit their function. Many viruses downregulate classical MHC class I to evade recognition by cytotoxic T cells, however this can release the brakes on NK cell activity, providing a second line host defence against infected cells. In rare circumstances HLA-E can present virus peptides to T cells and provoke a protective T cell response.

SARS-CoV-2 has caused one of the greatest global health challenges and while mass vaccination is bringing the pandemic under control there is an urgent need for new therapeutic approaches to treat this disease. We recently discovered HLA-E restricted CD8 T cell responses in SARS-CoV-2 infection that may synergise with classical T cell approaches in the control of virus replication and COVID-19 severity.

Virus replication is shaped by the cellular microenvironment and one important factor is local oxygen tension, where the hypoxia inducible transcription factors (HIFs) regulate transcriptional responses to low oxygen or hypoxia. We demonstrated that a hypoxic environment or treatment with HIF mimetic drugs inhibits SARS-CoV-2 entry and replication. Recent *in vivo* challenge studies show a role for HIFs to reduce the burden of infectious SARS-CoV-2 in the respiratory tract and inflammation-associated pathology in the lung. A defining feature of severe COVID-19 pneumonitis is systemic low oxygen (hypoxaemia), and we **hypothesize this hypoxic environment favours SARS-CoV-2 antigen presentation by HLA-E.** This project will examine the effect of hypoxia on the expression and transport of HLA-E in uninfected and SARS-CoV-2 infected lung epithelial cell lines and primary respiratory differentiated air-liquid interface cultures. Parallel experiments will study the efficacy of HLA-E restricted T cells to suppress SARS-CoV-2 replication under hypoxic conditions. We have access to a range of pharmacological agents that modify HIF activity to define underlying mechanisms.

These pathways are likely to impact other viruses and we would seek to translate our findings to study the impact of hypoxia on HLA-E presentation of HIV-1 peptides using existing and well characterized panel of HLA-E and classical HLA- restricted T cells. Elucidating the effect of hypoxia on HLA-E trafficking and antigen presentation provides new therapeutic opportunities to harness and regulate this under-studied arm of the innate immune response.

Training opportunities
This project will be co-supervised by Andrew McMichael and Jane McKeating who provide complementary expertise in viral T cell immunology, molecular virology and hypoxia biology. The interdisciplinary project will provide a unique training environment in viral immunology and range of techniques will be offered including in vitro models of T cell suppression of virus replication.

Transferable skills including oral presentations at joint lab meetings, critical reviewing of published scientific literature by contributing to journal clubs and scientific writing by reviewing and drafting manuscripts for publication.

Supervisors

Andrew McMichael is Emeritus Professor of Molecular Medicine at the University of Oxford. His research is focused on the role of T cell immunity in controlling virus infections. He has made discoveries on how T cells recognise virus peptides bound to HLA molecules on infected cells, how viruses escape from T cells by mutation and how HLA molecules are involved in innate immunity. He has also shown how T cell immunity can protect from infection and disease. He has written more than 500 papers, h-index 125, and is a Fellow of the Royal Society. He was until recently Director of the Weatherall Institute of Molecular Medicine and has been a scientific advisor for the Oxford CAMS partnership.

Jane McKeating is Professor of Molecular Virology at University of Oxford and her research studies the impact of hypoxia and circadian host signalling pathways on virus replication to uncover new therapies. Her laboratory has worked on clinical important viral pathogens including HIV, hepatitis B and C viruses and more recently SARS-CoV-2, with >200 papers (>31,673 citations, h-index 89). Jane holds a visiting Professorship at the Technical University of Munich (2015-present) and is a founding fellow of Reuben College.

Key references

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