CSC-COI SCHOLARSHIP PROPOSALS （2023）

CONTENTS

Primary supervisor: *Associate Prof. Geraldine Gillespie*

Co-supervisor: *Prof. Andrew McMichael*

Contact: geraldine.gillespie@ndm.ox.ac.uk

Primary supervisor: *Prof Tim Elliott*

Secondary supervisor: *Dr Pedroza-Pacheco*

Contact: tim.elliott@ndm.ox.ac.uk

Primary supervisor: *Dr Pablo Cespedes*

Secondary supervisor: *Prof. Michael L. Dustin*

Contact: pablo.cespedes@kennedy.ox.ac.uk

Primary supervisor: *Dr Pablo Cespedes*

Secondary supervisor: *Prof. Michael L. Dustin*

Contact: pablo.cespedes@kennedy.ox.ac.uk

Primary supervisor: *Prof. Sarah Rowland-Jones*

Secondary supervisor: *Dr Ester Gea-Mallorquí*

Contact: *sarah.rowland-jones@ndm.ox.ac.uk*

Primary supervisor: *Professor Marian Knight*

Secondary supervisor: *Dr Rema Ramakrishnan & Dr Joris Hemelaar*

Contact: marian.knight@npeu.ox.ac.uk

Supervisor: *Assoc Prof James Fullerton (Clinical)*

Co-supervisors: *Dr Roel De Maeyer (Non-clinical)*

Contact: *james.fullerton@ndorms.ox.ac.uk*

Supervisor: *Professor Geoffrey L. Smith*

Contact: *geoffey.smith@path.ox.ac.uk*

Project 1: Discovery of potent agonist peptides for tumour-reactive T cells

Primary Supervisor: *Ricardo A. Fernandes*

Additional Supervisors & Collaborators: *Tao Dong and Matthew Bottomley*

Contact: *ricardo.fernandes@ndm.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 (> 109) 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 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.

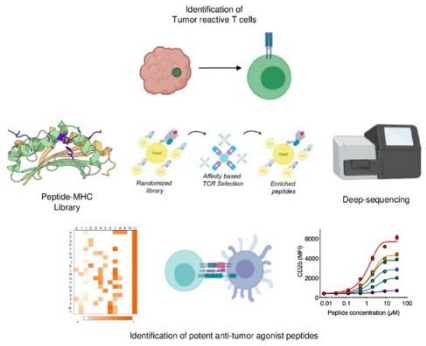

Figure 1. General overview of the experimental approach to discover peptide antigens to elicit robust anti-tumor T cell responses.

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 antigen peptides which will facilitate the identification, isolation and

activation of tumour-reactive T cells. Second, this information will guide the understanding of functional cross-reactivity of tumour-antigen reactive TCRs and the engineering of potent anti-tumour TCRs.

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 / Short Profile

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

5. Sušac L, Vuong MT, Thomas C, von Bülow S, O'Brien-Ball C, Santos AM, Fernandes RA, Hummer G, Tampé R, Davis SJ. Structure of a fully assembled tumor-specific T cell receptor ligated by pMHC. **Cell**. 2022 Aug 18;185(17):3201-3213.e19.

6. Yang X, Garner LI, Zvyagin IV, Paley MA, Komech EA, Jude KM, Zhao X, Fernandes RA, Hassman LM, Paley GL, Savvides CS, Brackenridge S, Quastel MN, Chudakov DM, Bowness P, Yokoyama WM, McMichael AJ, Gillespie GM, Garcia KC. Autoimmunity-associated T cell receptors recognize HLA-B*27-bound peptides. **Nature**. 2022 Dec;612(7941):771-777

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Project 2: Enhancing anti-tumor T cell function by controlled inhibition of checkpoint receptor signaling

Primary Supervisor: *Ricardo A. Fernandes*

Collaborators: Adan Pinto-Fernandez and REPRESSIT Consortium

Contact: *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 to expand this novel approach to target other key immune and cancer-specific receptors aimed at generating novel antitumor, RIPR-based, molecules.

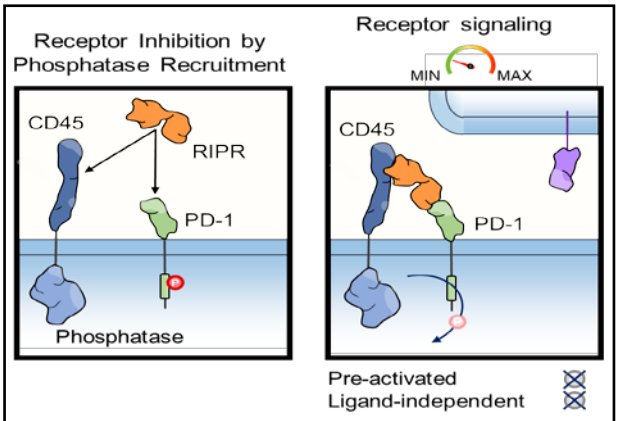

Fig. 1 | Receptor Inhibition by Phosphatase Recruitment. RIPR combines checkpoint blockade with CD45 recruitment. CD45 dephosphorylates receptors such as PD-1.

Development of RIPR-based molecules to inhibit immune checkpoint receptors.

This projects aims to develop novel RIPR proteins to target checkpoint receptors expressed in cytotoxic T cells, including CTLA-4, ILT2 and ILT4. Moreover, we will systematically test the potency of newly generated RIPR proteins using various anti-CD45 nanobodies. Newly generated molecules will be characterized in biophysical and in vitro stimulation assays. Binding on-rate, off-rate and affinity will be determined by surface plasmon resonance. After characterizing the binding properties of the RIPR molecules, their ability to potentiate T cell cytotoxic functions will be determined in vitro using co-culture assays with T cells and target cells. Markers of T cell activation will be quantified longitudinally using flow cytometry, western blotting and ELISAs. This comprehensive approach is expected to idenfity determinants of RIPR activity for various checkpoint receptors. This information will be used to guide the

design of future antagonists of checkpoint receptor signalling with strong potential for therapeutic applications.

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-nanobody libraries. T cell signalling assays will be used to determine the activity of newly generated RIPR molecules, providing 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 / Short Profile

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
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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

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Project 3: Hypoxia inducible factors: a therapeutic target for the treatment of Respiratory viral infections.

Primary Supervisor: *Jane McKeating*

Additional Supervisors & Collaborators: Peter Wing & Ling-Pei Ho

Contact: *jane.mckeating@ndm.ox.ac.uk*

Overview

Respiratory viral infections in humans are responsible for a significant proportion of global deaths, approximately 4.25 million/year, mostly in children and older adults. Respiratory syncytial virus (RSV) is the leading cause of infant hospitalisation worldwide, infecting approximately 34 million children each year and a major cause of morbidity and mortality in elderly and immunosuppressed adults. Although RSV infection is generally confined to the respiratory epithelium the resulting neutrophilic lung inflammation can be life-threatening. The only approved prophylactic treatment is Palivizumb (a neutralising anti-RSV Fusion antibody) and currently there are no approved vaccines, highlighting an urgent need for antiviral agents.

The COVID-19 pandemic has highlighted the importance of understanding fundamental host processes that viruses exploit to infect the respiratory tract. One important factor is local oxygen availability in the microenvironment that can activate hypoxia inducible factors (HIFs) that define the host response to low oxygen (hypoxia). HIFs activate the transcription of genes involved in cell metabolism and immune activity and are key regulators of host defences. We reported that HIFs inhibit SARS-CoV-2 infection and pathology and recent data shows their role in suppressing RSV infection. This project will use state-of-art RSV replication systems and animal models to examine the interplay between HIFs, RSV and immune cells with a focus on understanding their spatial relationship in respiratory tissue. We are keen to translate data from our experimental model systems to man and will study the relationship between HIFs, immature neutrophils and T cells in infected clinical samples using mass cytometry imaging methods. Comparative analysis of transcriptomic and immunotyping data from the experimental model systems with human clinical data will provide an opportunity to discover HIF driven immune regulatory pathways and this knowledge will inform the discovery of new therapies for the treatment of RSV.

Training Opportunities

The student will join a dynamic and lively team of biologists in the McKeating and Ho laboratories that bring complementary expertise in RSV biology, hypoxic signalling and T cell immunology. This interdisciplinary project will provide a unique training environment to gain expertise in super resolution imaging techniques to visualize RSV RNAs in complex tissues, digital spatial profiling and bio-informatic analysis of transcriptomic data sets. Transferable skills include oral presentations at joint lab meetings, critical review of published scientific literature by contributing to journal clubs and scientific writing by reviewing and drafting manuscripts for publication. The student will work in Nuffield Department of Medicine Research Building and MRC Human Immunology Unit and will have the opportunity to interface with a network of collaborators in Oxford, UK and internationally to translate their data to the wider biomedical.

Supervisor / Short Profile

Jane McKeating is Professor of Molecular Virology and her team studies the impact of hypoxia and circadian host signalling pathways on virus replication with the goal to uncover new therapies. Her laboratory works on human pathogens including hepatitis B and C viruses and more recently SARS-CoV-2 and RSV. Jane is a founding fellow of Reuben College, a new post-graduate Oxford college with a with a focus on interdisciplinary research addressing 21st century challenges.

Peter Wing is a CAMS-COI Fellow working with Jane McKeating who studies the interplay between virus

and hypoxic signalling pathways and their impact on disease outcome. Peter is the manager of the NDM research building containment level 3 laboratory to study viral pathogens at high containment.

Ling-Pei Ho is Professor of Respiratory Immunology, and her group works on the immune mechanisms underlying severe lung injury and aberrant lung repair. She has expertise in immun0pathology of severe viral infection (influenza and COVID-19) in murine and human lungs. Her group is based at the MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine.

Selected Publications

1. Mann ER *et al 2020. Longitudinal immune profiling reveals key myeloid signatures associated with COVID-19.* Sci Immunology 5(51):eabd6197. doi: 10.1126/sciimmunol.abd6197.
2. Wing PAC *et al* 2021. Hypoxic and pharmacological activation of HIF inhibits SARS-CoV-2 infection of lung epithelial cells. *Cell Rep* 35(3):109020. doi:10.1016/j.celrep.2021.109020.
3. Wing PAC *et al* 2022. Hypoxia inducible factors regulate infectious SARS-CoV-2, epithelial damage and respiratory symptoms in a hamster COVID-19 model. *PLoS Pathogens* 18(9):e1010807. doi: 10.1371/journal.ppat.1010807.
4. COMBAT Consortium et al 2022. A blood atlas of COVID-19 defines hallmarks of disease severity and specificity. *Cell* 185:916-38. doi: 10.1016/j.cell.2022.01.012.
5. Evans RA *et al* 2022. Clinical characteristics with inflammation profiling of Long COVID and association with one year recovery following hospitalization in the UK: a prospective observational study. *Lancet Respir Med* S213-2600 (22)00127-8.

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Project 4: Spatial exploration of hypoxic signalling and inflammation in hepatitis B associated liver cancer.

Primary Supervisor: *Jane McKeating*

Additional Supervisors & Collaborators: *Fadi Issa*

Contact: *jane.mckeating@ndm.ox.ac.uk*

Overview

Hepatitis B virus (HBV) is a global health challenge and major cause of liver disease and cancer. Hepatocellular carcinoma (HCC) is one of the fastest rising and most common causes of cancer-related deaths in the world. Treatment options depend on the disease stage and recent developments to boost the immune system against cancer have improved HCC outcomes for 20-30% of patients. A major challenge going forward is to understand why some patients respond to immunotherapy whilst others do not, and to use this knowledge to improve treatment. Low oxygen levels (hypoxia) in the liver potentiate HBV replication and allow tumour cells to multiply and hide from immune surveillance. One key hypoxic-regulated gene is carbonic anhydrase 9 (CA9) that is selectively expressed on cancer cells and induces an acidic environment that can suppress anti-cancer immune responses. This project will study archived liver cancer tissue from patients diagnosed with HBV or non-viral HCC and those receiving immunotherapy for CA9 expression. Digital spatial transcriptomic analysis will identify immune cell populations in the proximity of CA9+ tumours. We will focus on immunosuppressive regulatory T cells Tregs and will assess whether CA9 expression impacts on cell frequency, location, activation status and response to immunotherapy. Nearest neighbour analysis will examine cellular interactions at the micro-anatomic level. Our goal is understand why some patients respond to immunotherapies and others do not. This knowledge will inform future studies to evaluate CA9-targeted personalised therapies to sensitise HCC and improve treatment and survival.

Keywords: Spatial, hepatitis, Cancer, Inflammation, hypoxia.

Training Opportunities

The student will join a dynamic and lively team of biologists in the McKeating and Issa laboratories that bring complementary expertise in hepatitis B virology, hypoxic signalling and T cell immunology. This interdisciplinary project will provide a unique training environment to gain expertise in super resolution imaging techniques to visualize HBV RNA in complex tissues, digital spatial profiling and bio-informatic analysis of transcriptomic data sets. Transferable skills include oral presentations at joint lab meetings, critical review of published scientific literature by contributing to journal clubs and scientific writing by reviewing and drafting manuscripts for publication. The student will work in Nuffield Department of Medicine Research Building and Department of Surgical Sciences (John Radcliffe Hospital, Oxford) and will have the opportunity to interface with a network of collaborators in Oxford, UK and internationally to translate their data to the wider biomedical.

Supervisor / Short Profile

Jane McKeating is Professor of Molecular Virology and her team studies the impact of hypoxia and circadian host signalling pathways on virus replication with the goal to uncover new therapies. Her laboratory works on human pathogens including hepatitis B and C viruses and more recently SARS-CoV-2 and RSV. Jane is a founding fellow of Reuben College, a new post-graduate Oxford college with a with a focus on interdisciplinary research addressing 21st century challenges.

Fadi Issa is Associate Professor of Plastic Surgery and co-director of the Translational Research Immunology Group. He is interested in translational aspects of immunological research in cancer. transplantation, and autoimmunity. Our interests focus on manipulating the immune system with a view to treating immune-mediated diseases, with a particular interest in regulatory T cells.

Selected Publications

1. Wing PAC *et al* 2021. Hypoxia inducible factors regulate hepatitis B virus replication by activating the basal core promoter. *J Hepatol* 75(1):64-73. doi: 10.1016/j.jhep.2020.12.034.
2. Wing PAC *et al* 2021. Hypoxic and pharmacological activation of HIF inhibits SARS-CoV-2 infection of lung epithelial cells. *Cell Rep* 35(3):109020. doi:10.1016/j.celrep.2021.109020.
3. Wing PAC *et al* 2022. Hypoxia inducible factors regulate infectious SARS-CoV-2, epithelial damage and respiratory symptoms in a hamster COVID-19 model. *PLoS Pathogens* 18(9):e1010807. doi: 10.1371/journal.ppat.1010807.
4. Cross AR *et al* 2022. Spatial transcriptomics characterization of COVID-19 pneumonitis identifies immune circuits related to tissue injury. *JCI Insight*. doi:10.1172/jci.insight.157837.
5. Bottomley MJ *et al* 2022. Dampened Inflammatory Signalling and Myeloid-Derived Suppressor-Like Cell Accumulation Reduces Circulating Monocytic HLA-DR density and may associate with malignancy risk in long-term renal transplant patients. *Front Immunol* 13

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Project 5: (1)Switching on the embryonic globin genes to provide a new treatment for severe alpha thalassaemia; (2)Understanding how super-enhancers regulate gene expression; (3)Understanding the role of CTCF boundary elements in regulating gene expression

Primary Supervisor: *Professor Doug Higgs, FMedSci, FRS*

Contact: *DOUG.HIGGS@IMM.OX.AC.UK*

Project Overview

Our laboratory is interested in the general question of how mammalian genes are switched on and off during lineage commitment and differentiation. We use the most recent genomics technologies and computational approaches to study both the entire genome and individual genes in detail. We study all aspects of gene expression including the key cis-regulatory elements (enhancers, promoters and insulators), the transcription factors and co-factors that bind them, the epigenetic modifications of chromatin and DNA, and the role of associated phenomena such as chromosome conformation and nuclear sub-compartmentalisation using state-of-the-art imaging techniques. These studies are performed both in cell systems and in model organisms as well as in material from human patients with various inherited and acquired, genetic and epigenetic abnormalities. The translational goal of our work is to develop new ways to modify gene expression during blood formation with the aim of manipulating gene expression and ameliorating the clinical phenotypes of patients with a variety of blood disorders.

We study gene regulation using the human and mouse globin loci as haematopoietic cells undergo lineage fate decisions and differentiation. Our aim is to understand the principles by which all mammalian genes are switched on and off during cell fate decisions. Globin gene expression is controlled by a group of conserved, long-range regulatory elements some of which lie within the introns of an adjacent widely expressed gene (Nprl3) and another lies in intergenic DNA. All of these elements have the chromatin signature of enhancers. Using Chromosome Conformation Capture, we have shown that these enhancers physically interact with each other and with the globin gene promoters, and together are essential for normal globin gene expression. From genome-wide studies, this configuration appears to be a common feature of highly expressed, lineage-specific genes and such groups of regulatory elements are referred to as “super-enhancers”. We continue to study such enhancers to understand how they interact with the globin promoters and their effect on the transcription cycle. More recently we have developed analyses to examine gene regulation in single cells including imaging approaches that allow us to visualise chromatin movements and transcription of these genes in real time.

We have recently performed Hi-C experiments and have defined the Topologically Associated chromatin Domain (TAD) containing the globin gene cluster in erythroid and non-erythroid cells. We have also characterised the formation of this domain and of the enhancer promoter contacts during normal in vivo differentiation. We are currently investigating how activation, deletion and re-orientation of the globin regulatory elements (enhancers, promoters and boundary elements) affect expression of other genes within the same TAD and in neighbouring TADs. We also study chromatin structure and movement in real time using super-resolution imaging. Importantly, using globin as our model, we are addressing the general question of the relationship between higher order, long-range chromosomal structure and function.

In addition to understanding how genes are activated we are also interested in how they are silenced. One of the globin genes, lying within the TAD, is only expressed in early developmental life and then remains silenced during adult life. Reactivation of this gene may represent a novel therapeutic option for patients with severe alpha-thalassemia. We are studying the transcriptional and epigenetic pathway by which this gene is silenced and kept so even though it lies adjacent to active erythroid enhancers. Again, this is a general question in mammalian genetics and the globin system provides a unique opportunity to establish the biological principles by which gene silencing occurs.

An important aim of our work is to develop new ways of treating blood disorders by genome editing of the regulatory elements we are studying. We currently have clinical projects underway in Sri Lanka, China

and Thailand to develop such techniques to treat patients with thalassaemia, a common form of inherited anaemia.

Students joining our laboratory will have a choice of projects which address current topics in the regulation of gene expression, and their application to human genetic disease, using state-of-the-art approaches to these questions.

Training Opportunities

The Higgs laboratory offers a wide range of training opportunities in cell biology, molecular biology and computational approaches to biology. We train students in all aspects of cell biology using cell lines and primary cells from a range of organisms. We use all forms of flow cytometry to isolate and characterise common and rare cell types including stem/progenitor cells and we provide full training in this technology. When required, we also train students in mouse genetics to generate specific models for our research. Molecular techniques used in the laboratory include all forms of sequence- based analysis of DNA, RNA and chromatin both in cell populations (ATAC-seq, RNA-seq, ChIP-seq, CUT&RUN etc) and in individual single cells (scRNA-seq, scATAC-seq).

We also have access to the full range of proteomics, including single cell proteomics, and structural biology. The laboratory has also pioneered high resolution protocols for chromosomal conformation capture. We also routinely use genome editing, advanced forms of homology directed recombination, and synthetic biology to develop new models and approaches to understand the regulation of gene expression. An important new dimension to our research involves the use of advanced imaging, including super-resolution imaging, particularly in real time. Students interested in such projects will receive appropriate training in these techniques. We provide comprehensive training in all aspects of computational biology to analyse the resulting datasets.

Students are encouraged to attend the MRC Weatherall Institute of Molecular Medicine 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.

The host Department has a successful mentoring scheme, open to graduate students, which provides an additional possible channel for personal and professional development outside the regular supervisory framework. We hold an Athena SWAN Silver Award in recognition of our efforts to build a happy and rewarding environment where all staff and students are supported to achieve their full potential.

Supervisor / Short Profile

Douglas Higgs (FRS, DSc, FRCP, 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 Emeritus Professor of Molecular Haematology at the University of Oxford and an honorary consultant in the Department of Clinical Haematology (ORHA). Until 2020, he was Director of the MRC Molecular Haematology Unit (MHU) and Director of the Weatherall Institute of Molecular Medicine (WIMM). Douglas Higgs has published more than 300 primary research articles including many publications in the leading biological science journals (Nature, Science, Cell, Molecular Cell, Nature Genetics). In addition, he has produced and contributed to several standard textbooks in the field of haematology. He has supervised numerous undergraduate students, DPhil students and post-doctoral researchers, and many have gone on to have very successful independent careers. His research has elucidated many of the principles underlying normal gene expression and the mechanisms by which this is perturbed in human genetic disease. In addition, this

work forms the basis for the diagnosis and genetic counselling for the world's most common inherited form of anaemia (alpha thalassaemia).

Key References

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Project 6: SARS-CoV-2 replication, assembly, and egress

Primary Supervisor: *Prof. Peijun Zhang*

Contact: *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-FIB/SEM lamella preparation and volume imaging
- Data analysis and image reconstruction
- Computer molecular dynamics simulations

Supervisor / Short Profile

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. She joined the University of Pittsburgh School of Medicine in 2006 as an Assistant Professor became tenured in 2012. 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 Professor of Structural Biology 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.
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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):643-6. Featured on the cover of Nature.

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Project 7: Imaging HIV-1 nuclear import by in situ cryo-tomography and correlative microscopy

Primary Supervisor: *Prof. Peijun Zhang*

Contact: *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 unparalleled 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
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- Cryo-electron tomography and sub-tomogram averaging
- Correlative light and cryoEM imaging of virus infection
- Cryo-FIB/SEM lamella preparation
- Data analysis and image reconstruction
- Computer molecular dynamics simulations

Supervisor / Short Profile

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. She joined the University of Pittsburgh School of Medicine in 2006 as an Assistant Professor became tenured in 2012. 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 Professor of Structural Biology 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, Zhu Y, Yang Z, Xu C, Chaban Y, Nesterova T, Ning J, Böcking T, Parker MW, Monnie C, Ahn J, Perilla JR, **Zhang P*** (2021) Structure of native HIV-1 cores and their interactions with IP6 and CypA. *Sci Adv* 7(47):eabj5715.
3. 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.
4. 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: Development of machine learning approaches to integrate multi-dimensional, single cell and spatial proteomic data for cancer analytics in the human brain

Primary Supervisor: *Roman Fischer*

Additional Supervisors / Collaborators: *Philip Charles, Olaf Ansorge*

Contact: *roman.fischer@ndm.ox.ac.uk*

Project Overview

Glioblastoma multiforme (GBM) is a diverse, devastating and highly invasive brain cancer that can result in death after six months or less following diagnosis. The median survival is under two years with 40% of patients surviving year one and only 17% year two after diagnosis/treatment – a consequence of inevitable recurrence post-surgery and lack of any targeted disease modifying therapies. The genes, proteins and metabolites that drive GBM recurrence, invasion and growth especially in context of their localisation within the tumour are poorly understood, highlighting a clear need for improving understanding of the molecular mechanisms involved with the aim to improve targeted therapy and drug delivery at personalized level. The spatially resolved mapping of immune response, drug delivery and drug target distribution will facilitate the development of disease modifying therapies specifically targeted to the individual cancer.

Technologies that allow the spatially resolved detection of biomolecules are rapidly evolving with spatial proteome analysis in particular promising to allow molecular characterisation of an individual cancer and advancing drug/target discovery. In this project we will use data generated from novel spatial proteomics technology to map the deep proteome (>5000 proteins) and lipidome in GBM patient tissue down to single cell/type resolution, in order to establish the local molecular dependencies in GBMs at protein and lipid level. This project will require to apply and develop machine learning approaches to increase spatial resolution of the generated data based on integration with orthogonal spatial omics and visualisation techniques. Additionally, we aim to use machine learning to reduce the number sampling locations across the 3-dimensional space of tissue specimen.

The project will support the mapping of new disease markers and drug targets in dependence of their localisation in the fine structure of the tissue, map areas in which the immune system is active against the tumour and find new ways to trigger immune response and deliver targeted drugs to areas where they are needed.

The successful candidate will work at the interface of clinical research and pathology and will use data generated with state-of-the-art mass spectrometry platforms for lipidomic and proteomic analysis and will have the opportunity to work hands-on in the lab if desired. This can include sampling of brain tissue, data generation (spatial proteomics and lipidomics) and primary data analysis including spatially aware tools. The candidate will have clear focus of developing new approaches for machine learning based data imputation to increase spatial resolution and depth of generated data.

Approaches developed in this project will help discovering new drug targets and therapies in addition to establish novel technologies towards digital pathology and will advance machine learning approaches in drug development.

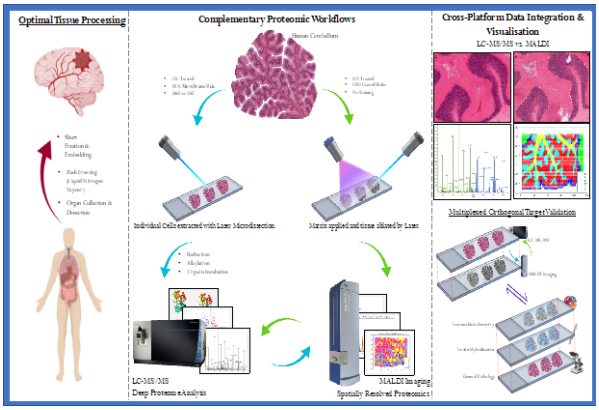

Figure 1 We have developed techniques (1,2) for mapping the deep proteome and lipidome in tissue combining Laser micro dissection with state-of-the-art mass spectrometry and MALDI imaging (3). Throughput and sensitivity are limiting factors in creating data with single cell spatial resolution (4). We aim to use machine learning based algorithms in order to address these limitations and to create 3 -dimensional atlases for tissue types, organs and tumours.

Training Opportunities

The candidate will have the opportunity to acquire highly transferable skills in mass spectrometry, proteomics, metabolomics with a clear focus on data analytics/integration and machine learning

- Multi-disciplinary 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
- Multi-omic data integration and visualisation
- Developing and applying spatially aware machine learning algorithms to create super resolution 3-dimensional proteome maps in tissue

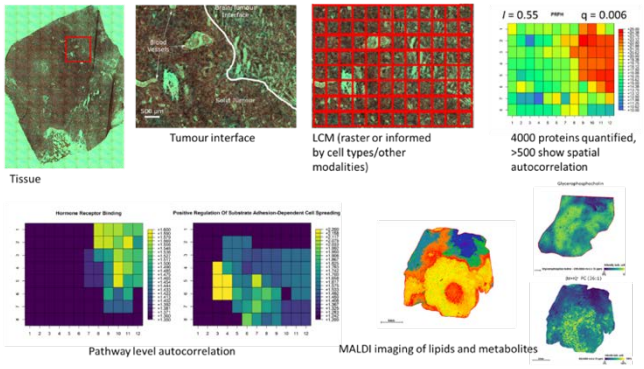

Figure 2 Established workflows (1,2) for state-of-the-art proteomics and MALDI imaging reveal (3) the spatial resolution of the cancer proteome in an AT/RT brain tumour and discover localized phenotypes at molecular and pathway level. This project aims to further advance this methodology for 3-dimensional visualisation of the deep cancer proteome within the spatial context of the tumour (i.e. glioblastoma) in order to discover druggable pathways in dependency of individual disease type and progression (real data shown).

Supervisor / Short Profile

Roman Fischer is an Associate Professor and Senior Group Leader in Clinical Proteomics at the University of Oxford. He leads the NDM Proteome Centre with laboratories at the Target Discovery Institute (TDI) and Wellcome Centre for Human Genetics (WHG) . RF studied Biotechnology at the Technical University Braunschweig and obtained his PhD at the Helmholtz Centre for Infection Research for the analysis of host-pathogen interactions of Listeria monocytogenes using proteomic methods (2007). After postdoctoral studies on Il-1 signalling in the laboratory of Professor Sir Philip Cohen in Dundee (Scotland), RF started to develop clinical proteomics at the University of Oxford in 2009. Since 2013 RF leads the Discovery Proteomics Facility and applies proteomic methods to a multitude of scientific questions (>150 peer-reviewed papers). Currently, RF focusses his research on the development of high-throughput and spatial proteomics methods and their application to large clinical cohorts and specimen.

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1. Davis S, Scott C, Ansorge O, Fischer R. Development of a Sensitive, Scalable Method for Spatial, Cell-Type-Resolved Proteomics of the Human Brain. J Proteome Res. 2019;18(4):1787-95.
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Project 9: Targeting Deubiquitylating Enzymes for Enhanced Cancer Immunotherapies

Primary Supervisor: *Dr. Adan Pinto Fernandez*

Additional Supervisors & Collaborators: *Prof. Benedikt Kessler* & *Prof. Tao Dong*.

Contact: *adan.pintofernandez@ndm.ox.ac.uk*

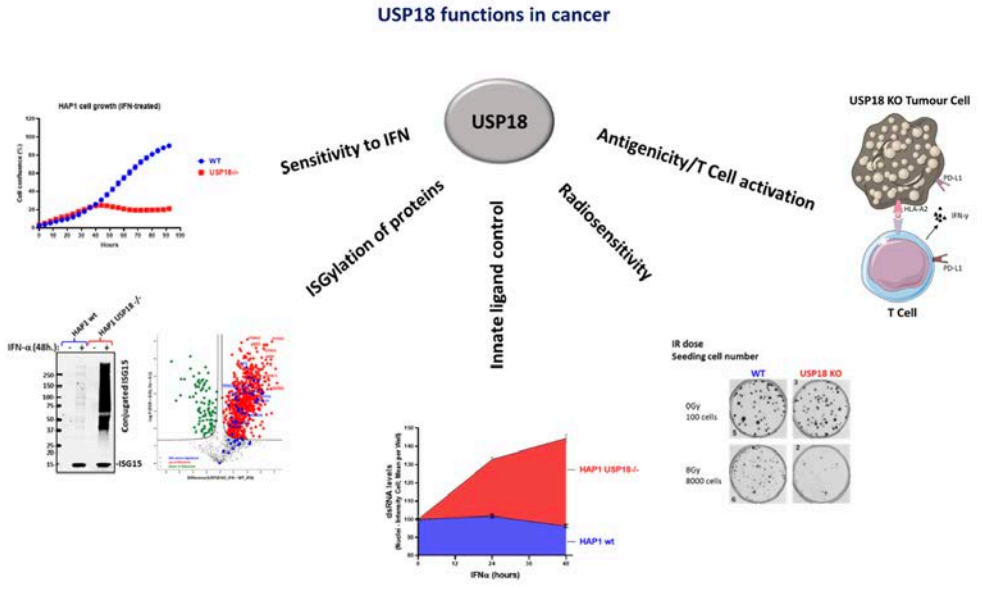
Project Overview

The proposed research involves the study of a class of druggable enzymes called deubiquitylating enzymes (DUBs) in cancer inflammation using advanced proteomics, ubiquitomics, lipidomics, and immunology techniques as main tools.

For instance, and following this approach, we have recently discovered that cancer cells lacking the DUB USP18, a negative regulator of the interferon pathway, are more antigenic and radiosensitive. At a molecular level, USP18-deficient cells accumulate innate immune ligands such as dsRNA, enhance the antigen presentation machinery, and hence they can activate more efficiently cytotoxic T cells, resulting in enhanced T cell killing and immunotherapy responses.

We will further study the translational potential of USP18 as a target boosting cancer immunotherapy and we will, in parallel, aim to identify and characterise additional regulators of the innate immune response with similar effects to USP18.

Roles of USP18 in cancer: Cancer cells lacking USP18 are sensitive to type-I interferon, accumulate ISGylated proteins and the innate ligand dsRNA, are radiosensitive and lead to a stronger functional T cell response.



Training Opportunities

We are experts in the study of the ubiquitin system in disease-relevant models using advanced ubiquitomics (GG-petidomics), activity-based protein profiling (ABPP), proteomics, lipidomics, chemical biology, cellular biology, and immunology techniques. These methodologies and matching data analysis approaches can be applied and learnt in our laboratory. Importantly, as part of the COI-NDM environment, we have access to innovative technology assuring the highest available standards in terms of data quality.

Finally, our laboratory has been always extremely interested in the translational aspect of our research and consequently, we have been involved in several collaborations with industry partners (including

Pfizer, Incyte, Xcellomics, ONO, FORMA therapeutics, and others). This provides an excellent opportunity to learn the complementary research dynamics happening in pharmaceutical companies.

Supervisor / Short Profile

Doctor Adán Pinto-Fernández is a Career Development Fellow at the CAMS Oxford Institute (COI) since November 2021 working on Ubiquitomics and Cancer Inflammation.

After finishing his degree in Biochemistry (Universidad de Oviedo; First-Class Honours), Adan started a PhD in Biochemistry at the University of Cantabria-CSIC (Piero Crespo's lab) studying the role of dimerisation of some elements of the Ras/MAPK signalling pathway in cancer models. After his PhD, and pursuing his interest in translational studies, he moved to Brussels to work in the laboratory of Prof. Oliver Feron (Université Catholique de Louvain). There he worked in the development of small molecules, specifically targeting hypoxic tumour cells, and he was involved in different studies trying to understand how tumour metabolism can be used as a pharmacological cancer target.

Prior to his current position at COI, Adan has been involved in targeting the ubiquitin system in cancer in the laboratory of Prof. Benedikt Kessler's at the University of Oxford. In particular, he has been studying deubiquitylating enzymes (DUBs) as cancer targets due to their ability to be inhibited by small molecules and to their involvement in biological processes related to cancer.

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. Epub 2020 Nov 20. PMID: 33214684; PMCID: PMC7884788.
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Project 10: Ubiquitomic and Proteomic Phenotyping of Malignant Pleural Effusions

Primary Supervisor: *Dr. Adan Pinto Fernandez*

Additional Supervisors & Collaborators: *Prof. Benedikt Kessler* & *Prof. Tao Dong*.

Contact: *adan.pintofernandez@ndm.ox.ac.uk*

Project Overview

The lungs are lined by a double layer of mesothelial cells the area in between these two branes is called the pleural space. Malignant pleural effusion (MPE) is the accumulation of excess fluid in the pleural cavity due to cancer and is associated with poor prognosis and deteriorated quality of life. Typical symptoms include breathlessness and dyspnoea, chest pain, cough, cachexia, and fatigue. Common malignancies that result in MPE include malignant pleural mesothelioma (MPM - the primary cancer of the pleural space), as well as malignancies, which can metastasise to the pleural space including lung and breast cancer, gynaecological malignancies, and lymphoma. MPE affects about 15% of cancer patients, with more than 150,000 cases in the United States and 100,000 cases in Europe each year.

The incidence of MPE is rising, due to the increasing prevalence of cancer and the improvements in survival of cancer patients. However, the median survival of MPE patients remains between 3 to 12 months from diagnosis. Despite advances in cancer treatments, clinical management of MPE remains largely palliative and aimed at symptom relief rather being curative. Management guidelines suggest draining only symptomatic effusions. To develop MPE therapies, a better understanding of the pleural space microenvironment is necessary.

We will use advanced mass-spectrometry proteomics and ubiquitomics to elucidate the molecular landscape of MPE patient samples, for both cells and fluids. The analysis of the proteome will allow us to identify differentially expressed proteins and pathways in the malignant samples, and thanks to the ubiquitomic analysis, we will focus on cancer-specific changes inside the ubiquitin system. Our group is interested in translational studies targeting deubiquitylating enzymes (DUBs), the proteases that revert ubiquitylation. For instance, and as a proof-of-concept, we have already collected and analysed the proteome of cancer cells present in the MPE of cancer patients. The results from this study can potentially identify disease biomarkers, including prognostic factors, and therapeutic targets.

Research overview: MPE samples from patients harbouring diverse types of malignancy will be analysed using established proteomic and ubiquitomic workflows, including total proteome, activity-based protein profiling (ABPP), and GG-peptidomics. Bioinformatics will help to identify potential biomarkers and druggable targets and to better understand the molecular landscape of the MPE environment. Hits will be validated, and functional assays will be performed to confirm the relevance of the identified hits.

Training Opportunities

We are experts in the study of the ubiquitin system in disease-relevant models using advanced ubiquitomics (GG-petidomics), activity-based protein profiling (ABPP), proteomics, lipidomics, chemical biology, cellular biology, and immunology techniques. These methodologies and matching data analysis

approaches can be applied and learnt in our laboratory. Importantly, as part of the COI-NDM environment, we have access to innovative technology assuring the highest available standards in terms of data quality.

Finally, our laboratory has been always extremely interested in the translational aspect of our research and consequently, we have been involved in several collaborations with industry partners (including Pfizer, Incyte, Xcellomics, ONO, FORMA therapeutics, and others). This provides an excellent opportunity to learn the complementary research dynamics happening in pharmaceutical companies.

Supervisor / Short Profile

Doctor Adán Pinto-Fernández is a Career Development Fellow at the CAMS Oxford Institute (COI) since November 2021 working on Ubiquitomics and Cancer Inflammation.

After finishing his degree in Biochemistry (Universidad de Oviedo; First-Class Honours), Adan started a PhD in Biochemistry at the University of Cantabria-CSIC (Piero Crespo's lab) studying the role of dimerisation of some elements of the Ras/MAPK signalling pathway in cancer models. After his PhD, and pursuing his interest in translational studies, he moved to Brussels to work in the laboratory of Prof. Oliver Feron (Université Catholique de Louvain). There he worked in the development of small molecules, specifically targeting hypoxic tumour cells, and he was involved in different studies trying to understand how tumour metabolism can be used as a pharmacological cancer target.

Prior to his current position at COI, Adan has been involved in targeting the ubiquitin system in cancer in the laboratory of Prof. Benedikt Kessler's at the University of Oxford. In particular, he has been studying deubiquitylating enzymes (DUBs) as cancer targets due to their ability to be inhibited by small molecules and to their involvement in biological processes related to cancer.

Dr Nikolaos Kanellakis Nikolaos is a Career Development Fellow at the China Oxford Institute where he leads the laboratory of Pleural Translational Research. His research interest and focus has been to phenotype and study patient derived specimens with the aim to understand pleural pathology and disease. In our lab we use functional genomics, transcriptomics and proteomics methods to elucidate pathogenesis pathways and associate these molecular patterns with clinical outcomes. He focusses to advance and expand precision medicine for pleural disease. Nikolaos has been involved in research projects focused on the development of biomarkers to improve patient stratification.

Professor Najib Rahman. Najib is the director of the Oxford Respiratory Trials Unit (ORTU). He is also leading clinical trials and the Oxford Pleural Disease Group. ORTU has a track record in design, delivery and analysis of trials in a number of areas of Respiratory Medicine including pleural disease, sleep apnoea, airways disease, and cancer. Najib's group conducts a diverse portfolio of research in pleural infection, undiagnosed pleural effusion, malignant pleural effusion, mesothelioma, pneumothorax, imaging and intervention. This work spans laboratory based translational work, early phase discovery science and practice changing clinical trials at oligo and multi-centre levels.

Key Publications

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Project 11: Using spatial transcriptomics and in vitro profiling to develop new cancer treatments through understanding the role of ageing fibroblasts in immune responses to cutaneous squamous cell carcinoma

Primary supervisor: *Dr Matthew Bottomley*

Secondary supervisor: *Prof Graham Ogg*

Contact: *matthew.bottomley@ndm.ox.ac.uk*

Project Overview

Introduction

Cutaneous squamous cell carcinoma (CSCC) is a major cause of ill-health in organ transplant recipients. Up to 40% of kidney transplant recipients (KTR) may develop CSCC over time, and up to half will develop another lesion within three years – CSCC is therefore the most common post-transplant malignancy [1]. Post-transplant CSCC exhibits a greater symptom burden and doubled metastatic risk compared to the general population. This leads to twice the cancer-specific mortality seen in immunocompetent cohorts.

The increased mortality due to post-transplant CSCC is partly due to inaccessibility to novel therapies, such as immune checkpoint blockade. Use of checkpoint inhibitors in transplant recipients leads to severe and irreversible transplant rejection in many.

Taken together, this means there is an unmet need to identify novel therapeutics for use in these populations, who suffer the greatest burden of disease.

The immune system plays a particularly important role in CSCC development and progression [2]. CSCC has the highest mutational burden of any malignancy, leading to multiple potential neoantigens that may drive a T cell response. Therefore, any perturbations of immune function may contribute to CSCC development and outcome. Conversely, this offers approaches to promote immune responses to cutaneous malignancy.

The immune system undergoes a series of changes with advancing age, with the hallmark features including reduced thymic output, chronic low-level inflammation ('inflamm-ageing') and a progressive shift amongst T cell proportions (particularly CD8+) from naïve to terminally differentiated [3]. Our previous work has shown that high levels of circulating markers of immune ageing ('immunosenescence') are an independent risk factor for CSCC development and outcomes [4].

Subsequent work, in preparation for publication, has demonstrated that advanced circulating immunosenescence is associated with reduced CD8+ T cell infiltration into CSCC in KTR but not immunocompetent controls (ICC). High-resolution spatial transcriptomic profiling has identified that this is associated with accumulation of macrophages with an immunoregulatory M2-like profile. Why these macrophages accumulate remains unclear, but may relate to chemotaxis mediated through CCR1.

Fibroblasts are an important component of the tumour microenvironment and signalling mediated through this population may play an important role in driving and modulating immune responses to cancer. Fibroblasts have previously been demonstrated to play a role in driving development of, and immunity to CSCC; genetic conditions that exhibit increased risk of CSCC are associated with alterations in fibroblast signalling to infiltrating leucocytes [5]. Aged keratinocytes can promote local senescence and inflammation through secretion of some, but not all, aspects of the senescence-associated secretory phenotype (SASP) [6].

From our previous data and the results presented above, we hypothesise that cancer-associated fibroblasts may be a source of CCR1-mediated signalling. Further, two key unanswered questions arise:

do aged fibroblasts differentially regulate the T cell response to CSCC? And does immunosuppression contribute to this process? This D.Phil project will explore both these questions.

Project outline

This project will utilise three main approaches.

Aim 1: Explore alterations in fibroblast density and infiltration of CSCC associated with immunosuppression and immune ageing

Firstly, matched CSCC collected and archived during a previous observational study of CSCC behaviour in KTR and ICC will be sectioned and analysed for fibroblast infiltration and density using immunofluorescence. Co-localisation with infiltrating CD68+ macrophages and CD3+ T cells will be evaluated. Comparison will be made between KTR and ICC and between those exhibiting more and less advanced circulating immunosenescence.

Aim 2: Explore changes in fibroblast-macrophage-T cell signalling in the tumour microenvironment with immunosuppression and immune ageing

Multiplex immunofluorescence will then be used to identify fibroblasts alongside infiltrating macrophages and T cells, prior to spatial transcriptomic profiling of co-localised fibroblasts, macrophages and T cells, using the Nanostring GeoMx platform. This allows highly-resolved, unbiased evaluation of gene expression in defined cell populations in histological sections, using hybridisation and next generation sequencing. Serial sections may be processed for spatial proteomic profiling in a similar manner.

Training in bioinformatic techniques will be provided, to facilitate the student's analysis of data arising from this approach. Ultimately, it is expected that pathways active in signalling between these two cell populations will be delineated and compared between groups as above.

Aim 3: Establish an in vitro model to validate aged fibroblast direct influence upon T cell behaviour

Dermal fibroblasts will be isolated from the abdominal skin of healthy controls exhibiting varying degrees of immunosenescence. These will be cultured as described previously and expression of relevant markers (identified in Aim 2) examined using appropriate multiplex gene expression panels on the Nanostring nCounter platform. Co-culture with SCC cell lines may be used to promote cancer-associated behaviour. Individual immunosuppressive agents such as calcineurin inhibitors may be added to cultured fibroblasts at physiological concentration to replicate the effect of the immunosuppressed milieu seen in transplant recipients and evaluate the effect upon fibroblast phenotype.

Cultured fibroblasts will then be added to autologous T cells at increasing concentrations and evaluated for their ability to suppress T cell responses to polyclonal stimuli in vitro. Where appropriate, blockade of pathway identified in Aim 2 by small molecule inhibition, RNA knockdown or monoclonal blocking antibody may be used to validate their importance.

Training Opportunities

This project offers the opportunity to develop:

- Skills in tissue handling, staining and analysis (Aims 1 and 2).
- Experience using the Nanostring GeoMx platform to undertake high-resolution spatial transcriptomic and proteomic profiling (Aim 2).
- Bioinformatic analysis of large transcriptomic or proteomic datasets
- Experience in handling human tissues and blood, cell isolation using density-gradient centrifugation, magnetic beads and/or fluorescence -assisted cell sorting (FACS) (Aim 3).
- Cell culture and T cell stimulation assays (Aim 3).
- RNA isolation and gene expression analysis using RT-PCR and the nCounter platform (Aim 3)

Supervisor / Short Profile

Dr Bottomley is a practicing nephrologist and, if the student were interested and made sufficient

academic progress, would be happy to act as sponsor for an observer contract with the Oxford University Hospitals NHS Foundation Trust to facilitate the student's periodic attendance in Dr Bottomley's nephrology clinic, allowing observation of British management of an unselected mix of patients with chronic kidney disease and those established on renal replacement therapy (including the long-term management of patients with a kidney transplant).

Primary Supervisor Profile

Dr Bottomley is a consultant nephrologist and Clinical Career Development Fellow at the CAMS-COI. He leads a research group investigating the impact of immune ageing and pharmacological immunosuppression upon peripheral T cell responses, with a particular focus upon skin.

He has a particular interest in understanding the risk factors and pathogenesis of skin malignancy (cutaneous squamous cell carcinoma, CSCC) relating to immunosuppression, with the aim of identifying new methods to identify kidney transplant recipients at high risk of CSCC and novel therapeutic pathways through which post-transplant CSCC may be managed and prevented. He has published previously on both clinical and biomarker-related aspect of CSCC prediction and management. He is a board member for the British Society for Skin Care in Immunosuppressed Individuals (BSSCII).

At CAMS-COI, Dr Bottomley is the lead for the Nanostring Hub, offering the full suite of platforms to facilitate bulk and spatial transcriptomic profiling of cells and tissues. He has extensive experience in the use of the GeoMx spatial transcriptomic profiling platform to deep profile immune networks in embedded tissue sections.

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3. Lee, K.A., et al., Immune Senescence, Immunosenescence and Aging. Front Aging, 2022. **3**: p. 900028.
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5. Goncalves-Maia, M., et al., NK Cell and Fibroblast-Mediated Regulation of Skin Squamous Cell Carcinoma Invasion by CLEC2A Is Compromised in Xeroderma Pigmentosum. J Invest Dermatol, 2020. **140**(9): p. 1723-1732.
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Project 12: Investigation of the role of viruses in pleural infection and associated respiratory disease

Primary supervisor: *Dr. Nikolaos Kanellakis & Prof. Peter Simmonds*

Secondary/additional supervisor: *Prof. Najib Rahman*

Contact: nikolaos.kanellakis@ndm.ox.ac.uk

Project Overview

Background: The pleural cavity is the area between the lung and chest wall. Established pleural infection (EPI) is a common and global disease where infected fluid develops in the pleural cavity.[1] Epidemiological data (Europe, USA) shows increasing incidence, especially in the elderly.[2-4] Clinical outcomes in pleural infection remain poor despite treatment.[1,5-8] Recent published studies demonstrate mortality of 20%, rising to 40% in vulnerable populations such as the elderly.[9] EPI represents a significant healthcare burden due to extended hospital admissions and potential requirement for surgical intervention.

In a recent study we investigated the bacteriology of EPI. Infections were found predominately polymicrobial with diverse bacterial frequencies observed in monomicrobial (dominated by Streptococcus pneumoniae) and polymicrobial disease (dominated by anaerobes and Gram negative).[10] Patient survival was greater in patients infected with anaerobes or bacteria of the Streptococcus anginosus group compared to those with Staphylococcus aureus. Predominant infection with Enterobacteriaceae was associated with higher risk of death.

The involvement of viruses in EPI remains to be investigated. Epidemiological data collected from English hospitals showed a correlation between laboratory confirmed influenza and incidence of EPI. A single centre cohort study reported that 24% (17/72) of EPI patients had serological evidence of recent influenza infection.

Aims: This translational project aims to comprehensively investigate the role of viruses in the establishment of pleural infection.

Materials and Methods: Pleural fluid specimens (n=243), collected for the largest observational study on pleural infection conducted to date will be used for virus screening. These samples are paired with blood specimens, clinical and radiological data. The samples will be subjected to metagenomic (target agnostic) next generation sequencing (NGS) to detect the presence of viruses. Pleural fluid specimens collected from patients with no history of EPI will be used as negative controls. NGS data will be processed using bioinformatic pipelines developed by the applicants to detect and assemble virus sequences from read data, allowing their identification, genetic characterization and semi-quantitation in the pleural fluid samples. Data emerging from parallel analyses of bacterial sequences will be compared with standard microbiology testing and the potential identification and detailed genetic characterization of bacteria refractory to standard isolation methods. Viral sequencing findings will be correlated with the bacteriology, clinical, and radiological data. This analysis will aim to identify the presence of viruses that correlate with one-year survival, hospital stay, and need for surgery.

Training Opportunities

The student will receive innovative and interdisciplinary education spanning scientific research, through a novel and personalized approach. In specific, the student will receive training on translational research, including research project design, next generation sequencing and associated bioinformatics, quantitative PCR and other molecular laboratory techniques, clinical sample handling, statistical data analysis and interpretation.

The University of Oxford provides a broad range of training courses workshops on how to analyse and present scientific data, and write scientific manuscripts (thesis, papers). Moreover, the DPhil student will take part in lab activities including lab meetings, journal clubs, seminars, and other events arranged by the departments and institutes across the University of Oxford. The DPhil student will have the

opportunity to present their work in international conferences and publish in peer reviewed journals.

Supervisor / Short Profiles

Dr Nikolaos Kanellakis: Nikolaos is a Career Development Fellow at the China Oxford Institute where he leads the laboratory of Pleural Translational Research. His research interest and focus has been to phenotype and study patient derived specimens with the aim to understand pleural pathology and disease. His lab uses functional genomics, transcriptomics and proteomics methods to elucidate pathogenesis pathways and associate these molecular patterns with clinical outcomes. He focusses to advance and expand precision medicine for pleural disease. Nikolaos has been involved in research projects focused on the development of biomarkers to improve patient stratification.

Professor Peter Simmonds: Peter's principal research interest and focus has been in virus evolution and epidemiology, emerging virus infections and molecular methods in virus detection and diagnosis. He is Unit Director of a recently awarded NIHR Blood Transfusion Research Unit (BTRU) research program into transfusion-transmitted infections (GEMS). This program is developing a range of molecular technologies for enhanced screening for virus infections, inclusion the application of large scale metagenomic high throughput sequencing approaches and associated bioinformatics, and enhanced virus genome detection methods such as CRISPR-Cas9/13 capture. These contribute to better characterization of human viromes and impacts on transplant safety and an evidence-based approach to assessment of residual infectious risk in blood and platelets components. These methodologies have wide application in clinical microbiology and virology contexts.

Clinical Supervisor

Professor Najib Rahman: Najib is the director of the Oxford Respiratory Trials Unit (ORTU). He is also leading clinical trials and the Oxford Pleural Disease Group. ORTU has a track record in design, delivery and analysis of trials in a number of areas of Respiratory Medicine including pleural disease, sleep apnea, airways disease, and cancer. Najib's group conducts a diverse portfolio of research in pleural infection, undiagnosed pleural effusion, malignant pleural effusion, mesothelioma, pneumothorax, imaging and intervention. This work spans laboratory based translational work, early phase discovery science and practice changing clinical trials at oligo and multi-center levels.

Key Publications

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2. JJC de Vries et al., Recommendations for the introduction of metagenomic next-generation sequencing in clinical virology, part II: bioinformatic analysis and reporting, *Journal of clinical virology* DOI: https://doi.org/10.1016/j.jcv.2021.104812
3. E. Bedawi, ERS/ESTS statement on the management of pleural infection in adults DOI: 10.1183/13993003.01062-2022
4. NI Kanellakis et al., The bacteriology of pleural infection (TORPIDS): an exploratory metagenomics analysis through next generation sequencing, *The lancet Microbe,* DOI: https://doi.org/10.1016/S2666-5247(21)00327-X
5. JP Corcoran at al., Prospective validation of the RAPID clinical risk prediction score in adult patients with pleural infection: the PILOT study, Eur Respir J., DOI: 10.1183/13993003.00130-2020

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3. Farjah F, Symons RG, Krishnadasan B, Wood DE, Flum DR. Management of pleural space infections: a population-based analysis. JThoracCardiovascSurg 2007;133:346-51.
4. Arnold DT. THE CHANGES IN INCIDENCE AND MANAGEMENT OF PLEURAL EMPYEMA IN ENGLAND OVER THE LAST DECADE. Thorax 2019;74:A9.

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8. Nielsen J, Meyer CN, Rosenlund S. Outcome and clinical characteristics in pleural empyema: a retrospective study. Scand J Infect Dis 2011;43:430-5.
9. Ferguson AD, Prescott RJ, Selkon JB, Watson D, Swinburn CR. The clinical course and management of thoracic empyema. QJM 1996;89:285-9.
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Project 13: Understanding aetiology, pathogenesis and progression to pleural infection through ex vivo phenotyping.

Primary supervisor: *Dr. Nikolaos Kanellakis & Prof. Peter Simmonds*

Secondary/additional supervisor: *Prof. Najib Rahman*

Contact: nikolaos.kanellakis@ndm.ox.ac.uk

Project Overview

Background: The lungs are lined by a double layer of mesothelial cells. The area in-between these two membranes is called the pleural cavity. Established Pleural Infection (EPI), where infected fluid develops between the lung and chest wall, is a common and global disease.[1] Epidemiological data (Europe, USA) shows increasing incidence, especially in the elderly.[2-4] Clinical outcomes in pleural infection remain poor despite treatment.[1,5-8] Recent published studies demonstrate mortality of 20%, rising to 40% in vulnerable populations such as the elderly.[9] Pleural infection develops as a complication of pneumonia and approximately 57% of these patients develop a pleural effusion termed as Uncomplicated Parapneumonic Effusion (UPE).[10-12] The majority of these effusions have no evidence of frank infection, but 15% of patients with effusion and pneumonia will later display evidence of EPI[12]. The exact molecular pathway of disease progression remains unknown.

Animal data suggests that the presence of pleural effusion is a necessary substrate for sustained pleural infection - direct inoculation of bacteria into the pleural cavity in the absence of effusion results in either overwhelming sepsis or spontaneous recovery, and not sustained bacterial growth [13,14]. A clinical study has demonstrated that patients with pneumonia and radiologically identified pleural effusion have poorer outcomes than those without effusion (with EPI excluded)[15]. This suggests that the pathological processes resulting in UPE formation associates with worse prognosis. Understanding formation of fluid and progression of UPE to EPI are vital to improve clinical management.

Aims: This is a translational ex vivo phenotyping project with the aims to investigate how and why EPI becomes established and progresses, and to identify potential novel treatment targets and methods to stratify patients in a precise, data driven manner.

Approach: To achieve the above aims, we will prospectively collect pleural fluid and blood specimens, as well as oral cavity swabs from patients at two timepoints, a) when they present with UPE and b) when they progress to EPI. In addition to samples, we will collect radiological (CT, ultrasound) and clinical data including length of hospital stay, requirement for further pleural interventions (drain insertion, surgery, intensive care) death, functional status (lung function, exercise ability). We will investigate the differences in intrapleural immune and fibrinolytic microenvironments. Moreover, we will compare the oral and pleural fluid microbiology for the two timepoints. Host cells isolated from these samples will be subjected to single-cell multi-omics profiling (i.e. transcriptomic, epigenomic and proteomic quantification). Proteomic measurements will be derived from a panel of 30 antibody-oligonucleotide conjugates (BD AbSeq) tagging common immune markers, while transcriptomic measurements will be obtained using poly A-based single-cell RNA-sequencing and epigenomic from assaying chromatin accessibility (ATAC-seq). These layers of information will be integrated to identify and annotate immune cell populations, their biological signatures and key mediators of response. We will relate findings to the time course of disease, define predictive biomarkers of response states and validate potential targets using genome editing and related approaches. In addition to characterizing the composition of pleural fluid, the availability of paired blood samples within this study will enable us to track the migration of different immune cell types between blood and the pleural cavity.

Training Opportunities

The University of Oxford provides a broad range of training courses workshops on how to analyse and present scientific data, and write scientific manuscripts (thesis, papers). Moreover, the DPhil student will take part in lab activities including lab meetings, journal clubs, seminars, and other events arranged by the departments and institutes across the University of Oxford. The DPhil student will have the

opportunity to present their work in international conferences and publish in peer reviewed journals.

Supervisor / Short Profiles

Professor Julian Knight: Julian's scientific interest and focus has been to understand how genetic and genomic variation impacts genes and processes critical to mounting an appropriate immune response and may contribute to susceptibility to infectious, inflammatory and autoimmune diseases. His lab aims to leverage recent advances in human genetics and genomics to improve understanding of biological processes in immune disease pathogenesis, validate drug targets and advance opportunities for precision medicine. Julian's work combines bioinformatics with functional genomic approaches in primary cells in disease relevant contexts and establishing mechanism. His work promotes use of genomics for drug target identification and validation, public engagement with genomics and implementation of genomic medicine in the clinic through education, training and a multidisciplinary approach.

Dr Nikolaos Kanellakis: Nikolaos is a career development fellow at the China Oxford Institute where he leads the laboratory of Pleural Translational Research. His research interest and focus has been to phenotype and study patient derived specimens with the aim to understand pleural pathology and disease. In our lab we use functional genomics, transcriptomics and proteomics methods to elucidate pathogenesis pathways and associate these molecular patterns with clinical outcomes. He focusses to advance and expand precision medicine for pleural disease. Nikolaos has been involved in research projects focused on the development of biomarkers to improve patient stratification.

Clinical Supervisor

Professor Najib Rahman: Najib is the director of the Oxford Respiratory Trials Unit (ORTU). He is also leading clinical trials and the Oxford Pleural Disease Group. ORTU has a track record in design, delivery and analysis of trials in a number of areas of Respiratory Medicine including pleural disease, sleep apnea, airways disease, and cancer. Najib's group conducts a diverse portfolio of research in pleural infection, undiagnosed pleural effusion, malignant pleural effusion, mesothelioma, pneumothorax, imaging and intervention. This work spans laboratory based translational work, early phase discovery science and practice changing clinical trials at oligo and multi-center levels.

Key publications

1. Cano-Gamez E, Burnham KL, Goh C, Malick ZH, Kwok A, Smith DA, Peters-Sengers H, Antcliffe D, Investigators G, McKechnie S, Scicluna BP, van der Poll T, Gordon AC, Hinds CJ, Davenport EE & Knight JC (2022). An immune dysfunction score for stratification of patients with acute infection based on whole blood gene expression. Science Translational Medicine 10.1126/scitranslmed.abq4433
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5. E. Bedawi, ERS/ESTS statement on the management of pleural infection in adults Eur Respir J., DOI: 10.1183/13993003.01062-2022

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4. Arnold DT. THE CHANGES IN INCIDENCE AND MANAGEMENT OF PLEURAL EMPYEMA IN ENGLAND OVER THE LAST DECADE. Thorax 2019;74:A9.
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Project 14: The role of lipid mediated immune responses in pleural infection

Primary supervisor: *Dr Yi-Ling Chen & Dr Nikolaos Kanellakis*

Secondary/additional supervisor: *Prof. Najib Rahman & Prof. Graham Ogg*

Contact: yi-ling.chen@rdm.ox.ac.uk

Project Overview

Background: The pleural cavity is the area between the lung and chest wall. Established pleural infection (EPI) is a common and global disease where infected fluid develops in the pleural cavity.[1] Recent studies have reported an increasing incidence of EPI, and a mortality rate of 20% that rises to 40% in vulnerable populations, such as the elderly.[2-5] Clinical outcomes in EPI remain poor despite treatment; therefore, it represents a significant healthcare burden due to extended hospital admissions and potential requirement for surgical intervention.[1, 6-9]

CD1 molecules are a group of nonpolymorphic MHC-I like antigen-presenting proteins and can present a broad variety of lipid antigens, ranging from exogenous lipids to endogenous mammalian self-lipids, to T cells.[10] In humans, four types of CD1 molecules (CD1a, CD1b, CD1c and CD1d) are express on the surface of antigen-presenting cells (APCs). Under steady-state conditions, CD1 continuously sample the environment, but can also be modulated by inflammation and infection, impacting the pathogenesis of some diseases.[11-15]

Alterations of lipid metabolism pathways is often observed in individuals affected by infection, including EPI.[16, 17] In addition, recognition of self and bacterial lipids by CD1-restricted T cells has been implicated in the development of several infectious and inflammatory diseases.[11, 18, 19] However, Whether the abnormal lipid profiles play a role in modifying CD1-reactive T cell functions is hitherto understudied. Moreover, the changes in lipid content of EPI fluid remain unexplored.

Aims: Here, we propose a project to decipher the effect of aberrant lipid profiles on CD1-restricted T cells responses in EPI. This study will elucidate the underlying mechanisms of host-pathogen interaction, which may prompt an evaluation of promising new therapeutic targets toward activating or dampening immune responses.

Materials and Methods: This is a translational project and patient derived samples will be used. In specific, we will collect pleural fluid, and blood specimens from patients with and without confirmed EPI, the latter will be used as negative controls. The PBMC and pleural fluid mononuclear cell (PFMC) will be cryopreserved for phenotypic analysis, and the plasma and pleural fluid will be stored for soluble factor profiling.

Approaches*:*

1) **Identification of CD1-reactive T cells in PBMC and PFMC from EPI patients.**

   CD1-expressing artificial antigen presenting cells (aAPCs) will be used to present endogenous lipid antigens to polyclonal T cells isolated from the PBMCs and PFMCs from patients with or without confirmed EPI. Relevant cytokine productions from T cells will be identified using ELISpot or cytokine-capture assays. In addition, CD1 tetramers will be used to monitor the CD1-reactive T cells distribution and frequency in the *ex vivo* specimens.

2) **Characterisation of the soluble factors in pleural fluid.**

   Bead-based multiplex assay panels will be used to identify the constitution and concentrations of relevant cytokines, chemokines, and immune checkpoint proteins involved. Correlations between the pattern of soluble factors, pathogens identified, and phenotypes of CD1-reactive T cells from the same cohort will be analysed to identify prognostic potentials.

3) **10X Single Cell Immune Profiling and bioinformatic analysis of cells derived from EPI patients.**

PBMCs and PFMCs harvested from patients with or without confirmed EPI will be subjected to single cell CITE-seq and T-cell receptor (TCR) repertoire analysis. This will prompt the understanding of complex interactions among different immune cells and pathogens, as well as T cell antigen specificity, in the development of EPI. Collectively, network analysis of clinical metadata, pathogen composition, soluble factor profiles, and transcriptomic and surface protein expression of immune infiltrates will advance the understanding of immune involvement in EPI.

Training Opportunities

To identify CD1-reactive T cells in human peripheral blood and pleural fluid samples, the scholar will learn sterile tissue culture techniques to handle cell lines and primary cell cultures. To investigate the functions of immune cells, they will be familiarised with essential immunology techniques, such as ELISpot, Flow cytometer, Bead-based multiplex assays and molecular biology and protein purification methods, etc. Scholar will have the possibility of generating large single cell data from cells isolated from EPI samples and involving in the bioinformatic analysis.

The University provides a broad range of resources and workshops on how to develop a coherent narrative and good presentation skills, and guides on thesis writing. In addition, the scholar will take part in the activities such as lab meetings, journal clubs, seminars, and other activities arranged by the departments and institutes across the university, as well as attend relevant courses to build their knowledge. They will also have opportunities to present their work in lab meeting and/or conferences, and draft manuscripts for publication.

Supervisor / Short Profiles

Primary supervisors:

Dr Yi-Ling Chen. Yi-Ling is a Career Development Fellow at the China Oxford Institute. Her research has been focussed on the innate lymphocyte mechanisms under cutaneous inflammatory conditions. Her work furthers the understanding of the roles of commensal and pathogen responses in tissue homeostasis and microbial defence at the cellular and molecular level, with specific interests in the involvement of lipid-reactive T cells. With high dimensional transcriptomics and T cell clonality analysis approaches, her studies have progressed translationally towards the evaluation of promising new therapeutic targets and the discovery of new lipid candidates for immune intervention.

Dr Nikolaos Kanellakis. Nikolaos is a Career Development Fellow at the China Oxford Institute where he leads the laboratory of Pleural Translational Research. His research interest and focus has been to phenotype and study patient derived specimens with the aim to understand pleural pathology and disease. His lab uses functional genomics, transcriptomics and proteomics methods to elucidate pathogenesis pathways and associate these molecular patterns with clinical outcomes. He focusses to advance and expand precision medicine for pleural disease. Nikolaos has been involved in research projects focused on the development of biomarkers to improve patient stratification.

Clinical supervisor

Professor Najib Rahman. Najib is the director of the Oxford Respiratory Trials Unit (ORTU). He is also leading clinical trials and the Oxford Pleural Disease Group. ORTU has a track record in design, delivery and analysis of trials in a number of areas of Respiratory Medicine including pleural disease, sleep apnea, airways disease, and cancer. Najib's group conducts a diverse portfolio of research in pleural infection, undiagnosed pleural effusion, malignant pleural effusion, mesothelioma, pneumothorax, imaging and intervention. This work spans laboratory based translational work, early phase discovery science and practice changing

Key collaborator

Professor Graham Ogg. Graham's research has been aiming to understand the role of human cutaneous immune responses in mechanisms of disease, treatment and vaccination. The work proposed here will build on the lab's existing CD1 work (Chen et al. in revision; Hardman, C.S., et al.[20])

Key Publications

1. NI Kanellakis et al., The bacteriology of pleural infection (TORPIDS): an exploratory metagenomics analysis through next generation sequencing. Lancet Microbe, 2022. 3(4): p. e294-e302. DOI: https://doi.org/10.1016/S2666-5247(21)00327-X
2. E. Bedawi, ERS/ESTS statement on the management of pleural infection in adults. Eur Respir J, 2022. DOI: 10.1183/13993003.01062-2022
3. Chen, Y.L., et al., Re-evaluation of human BDCA-2+ DC during acute sterile skin inflammation. J Exp Med, 2020. 217(3): p. jem.20190811. DOI: 10.1084/jem.20190811
4. Hardman, C.S., et al., CD1a promotes systemic manifestations of skin inflammation. Nat Commun, 2022. 13(1): p. 7535. DOI: 10.1038/s41467-022-35071-1
5. Monnot, G.C., et al., Staphylococcal phosphatidylglycerol antigens activate human T cells via CD1a. Nat Immunol, 2023. 24(1): p. 110-122. DOI: 10.1038/s41590-022-01375-z

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1. Cargill, T.N., et al., A systematic review of comorbidities and outcomes of adult patients with pleural infection. Eur Respir J, 2019. 54(3).
2. Grijalva, C.G., et al., Emergence of parapneumonic empyema in the USA. Thorax, 2011. 66(8): p. 663-668.
3. Farjah, F., et al., Management of pleural space infections: a population-based analysis. J.Thorac.Cardiovasc.Surg., 2007. 133(2): p. 346-351.
4. Arnold, D.T., The changes in incidence and management of pleural empyema in England over the last decade Thorax, 2019. 74: p. A9.
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11. Siddiqui, S., L. Visvabharathy, and C.R. Wang, Role of Group 1 CD1-Restricted T Cells in Infectious Disease. Front Immunol, 2015. 6: p. 337.
12. de Jong, A., et al., CD1a-autoreactive T cells are a normal component of the human alphabeta T cell repertoire. Nat Immunol, 2010. 11(12): p. 1102-9.
13. Bourgeois, E.A., et al., Bee venom processes human skin lipids for presentation by CD1a. J Exp Med, 2015. 212(2): p. 149-63.
14. Jarrett, R., et al., Filaggrin inhibits generation of CD1a neolipid antigens by house dust mite derived phospholipase. Science Translational Medicine, 2016. 8: p. 325ra18.
15. Cheung, K.L., et al., Psoriatic T cells recognize neolipid antigens generated by mast cell phospholipase delivered by exosomes and presented by CD1a. J Exp Med, 2016. 213(11): p. 2399-2412.
16. Saballs, M., et al., HDL-c levels predict the presence of pleural effusion and the clinical outcome of community-acquired pneumonia. Springerplus, 2016. 5(1): p. 1491.
17. Bagchi, S., S. Genardi, and C.R. Wang, Linking CD1-Restricted T Cells With Autoimmunity and Dyslipidemia: Lipid Levels Matter. Front Immunol, 2018. 9: p. 1616.
18. Prigozy, T.I. and M. Kronenberg, Presentation of bacterial lipid antigens by CD1 molecules. Trends in Microbiology, 1998. 6(11): p. 454-459.

19. Monnot, G.C., et al., Staphylococcal phosphatidylglycerol antigens activate human T cells via CD1a. Nat Immunol, 2023. 24(1): p. 110-122.

20. Hardman, C.S., et al., CD1a promotes systemic manifestations of skin inflammation. Nat Commun, 2022. 13(1): p. 7535.

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Project 15: Study of antibody responses against emerging pathogens toward vaccine design, therapeutics and protection.

Primary supervisor: *Prof. Gavin Screaton*

Co-supervisor: *Dr. Juthathip Mongkolsapaya*

Contact: gavin.screaton@medsci.ox.ac.uk

Project Overview

Background: Emerging pathogens can cause severe deadly diseases when they are introduced into naive populations. Emerging diseases include dengue virus, zika virus, HIV, Ebola virus, SARS and MERS and, the most recent, COVID. They caused severe disease outbreaks which can turn into pandemics because we lack control tools such as diagnostic tests, therapeutics and vaccines. COVID is the clear example of how a new pathogen emerging in Wuhan could cause a pandemic leading to millions of deaths and effecting global economy. It is inevitable that there will be new pathogen emergence. Therefore, it is important to prepare for new epidemic/pandemic threats.

Our lab has studied a number of emerging viruses, dengue, Zika, SARS CoV-2 and Ebola for more than 20 years. Our work had contributed to understanding pathogenic mechanisms, to generate diagnostic and therapeutic reagents, design vaccines, and contributed to policy development. We have published several scientific articles in high impact journals such as Nature, Science, Cell, Nature immunology, Nature Communication, and Immunity. For example, during the COVID pandemic, we generated reagents that were involved in establishing a protocol to measure the antibody response, which have been used to monitoring the immune status in UK population. We have generated hundreds of monoclonal antibodies from infected- and vaccinated- individuals. We characterised their neutralisation activities, cross-reaction among the variants, and bio-physical properties. And in combination with crystal and cryo-EM structures, we described the antigenic distance among the variants and how new emerging variants escaped from existing immunity (figure 1).

We also demonstrate how new variants effect the neutralising abilities of vaccinated individuals. For example, when BA.4/5 emerged, we studied the neutralization of BA.4/5 using a range of vaccine and naturally immune serum and panels of monoclonal antibodies. BA.4/5 shows reduced neutralization by the serum from individuals vaccinated with triple doses of AstraZeneca or Pfizer vaccine compared with BA.1 and BA.2 (figure 2). Furthermore, using the serum from BA.1 vaccine breakthrough infections, there are, likewise, significant reductions in the neutralization of BA.4/5, raising the possibility of repeat Omicron infections.

Aim: The general aim of the proposed D.Phil project is to apply our expertise to study new emerging pathogens such as Sudan Ebolavirus which caused a large outbreak in Uganda at the end of 2022.

Training Opportunities

The student will join a team having more than 20-year experience in virology, immunology and molecular biology. Our work has made a great contribution to the field with a number of high impact publications. The student will be trained by experienced post-docs in a broad range of techniques such as basic virology (viral isolation, viral propagation, neutralisation and viral titration), generation of pseudovirus, 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 structural analysis to design new immunogens.

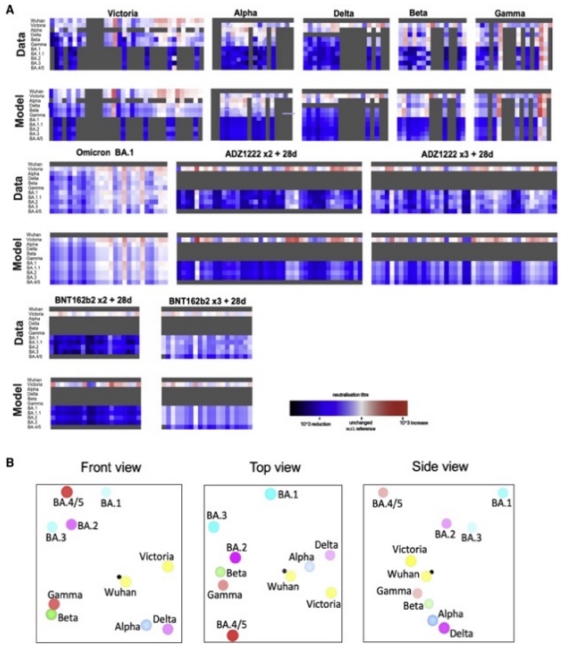

Figure 1 A) Neutralization data and model (log titer values) used to calculate antigenic maps in (B). Columns represent sera collected from inoculated volunteers or infected patients. Rows are challenge strains: Victoria, Alpha, Delta, Beta, Gamma, BA.1, BA1.1, BA.2, BA.3, and BA.4/5 in order. Values are colored according to their deviation from the reference value. The reference value is calculated on a serum-type basis as the average of neutralization titers from the row that gives this the highest value.

(B) Orthogonal views of the antigenic map showing BA.4/5 in the context of the positions of previous VoC and BA.1, BA.1.1, BA.1, and BA.2, calculated from pseudovirus neutralization data. Distance between two positions is proportional to the reduction in neutralization titer when one of the corresponding strains is challenged with a serum derived by infection by the other. No scale is provided since the figures are projections of a three-dimensional distribution; however, the variation can be calibrated by comparison with (i) BA.1 to BA.2, which is 2.93× reduced, and (ii) BA.2 to BA.4/5, which is 3.03× reduced. The third dimension may be inferred by fading of the colors with greater distance from the viewer.

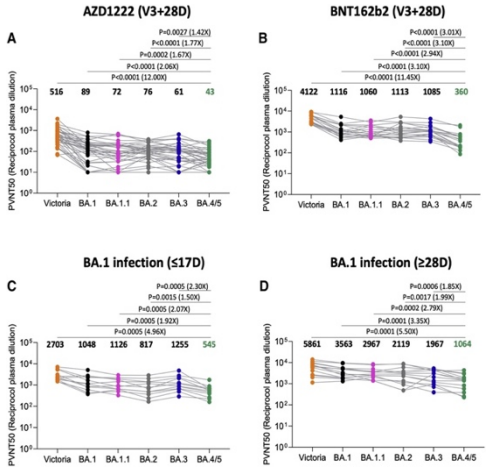

Figure 2 (A and B) IC50 values for the indicated viruses using serum obtained from vaccinees 28 days following their third dose of vaccine (A) AstraZeneca AZD1222 (n = 41) or (B) 4 weeks after the third dose of Pfizer BNT162b2 (n = 19).

(C and D) Serum from volunteers suffering breakthrough BA.1 infection taken (C) early, i.e., ≤17 days from symptom onset (median 12 days) n = 12 and (D) late, i.e., ≥28 days from symptom onset (median 45 days) n = 14. Comparison is made with neutralization titers to Victoria an early pandemic strain, BA.1, BA.1.1, BA.2, and BA.3. Geometric mean titers are shown above each column. The Wilcoxon matched-pairs signed-rank test was used for the analysis, and two-tailed p values were calculated

Key Publications

1. Tuekprakhon, A. et al. Antibody escape of SARS-CoV-2 Omicron BA.4 and BA.5 from vaccine and BA.1 serum. Cell 185, 2422-2433 e2413, doi:10.1016/j.cell.2022.06.005 (2022).
2. Nutalai, R. et al. Potent cross-reactive antibodies following Omicron breakthrough in vaccinees. Cell 185, 2116-2131 e2118, doi:10.1016/j.cell.2022.05.014 (2022).
3. Dejnirattisai, W. et al. SARS-CoV-2 Omicron-B.1.1.529 leads to widespread escape from neutralizing antibody responses. Cell 185, 467-484 e415, doi:10.1016/j.cell.2021.12.046 (2022).
4. Dejnirattisai, W. et al. Reduced neutralisation of SARS-CoV-2 omicron B.1.1.529 variant by post-immunisation serum. Lancet 399, 234-236, doi:10.1016/S0140-6736(21)02844-0 (2022).
5. Sharma, A. et al. The epitope arrangement on flavivirus particles contributes to Mab C10's extraordinary neutralization breadth across Zika and dengue viruses. Cell 184, 6052-6066 e6018, doi:10.1016/j.cell.2021.11.010 (2021).
6. Liu, C. et al. Reduced neutralization of SARS-CoV-2 B.1.617 by vaccine and convalescent serum. Cell 184, 4220-4236 e4213, doi:10.1016/j.cell.2021.06.020 (2021).

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8. Dejnirattisai, W. et al. The antigenic anatomy of SARS-CoV-2 receptor binding domain. Cell 184, 2183-2200 e2122, doi:10.1016/j.cell.2021.02.032 (2021).
9. Supasa, P. et al. Reduced neutralization of SARS-CoV-2 B.1.1.7 variant by convalescent and vaccine sera. Cell 184, 2201-2211 e2207, doi:10.1016/j.cell.2021.02.033 (2021).
10. Zhou, D. et al. Evidence of escape of SARS-CoV-2 variant B.1.351 from natural and vaccine-induced sera. Cell 184, 2348-2361 e2346, doi:10.1016/j.cell.2021.02.037 (2021).
11. Slon-Campos, J. L. et al. A protective Zika virus E-dimer-based subunit vaccine engineered to abrogate antibody-dependent enhancement of dengue infection. Nat Immunol 20, 1291-1298, doi:10.1038/s41590-019-0477-z (2019).
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13. Renner, M. et al. Characterization of a potent and highly unusual minimally enhancing antibody directed against dengue virus. Nat Immunol 19, 1248-1256, doi:10.1038/s41590-018-0227-7 (2018).
14. 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).
15. Dejnirattisai, W. et al. Dengue virus sero-cross-reactivity drives antibody-dependent enhancement of infection with zika virus. Nat Immunol 17, 1102-1108, doi:10.1038/ni.3515 (2016).
16. Rouvinski, A. et al. Recognition determinants of broadly neutralizing human antibodies against dengue viruses. Nature 520, 109-113, doi:10.1038/nature14130 (2015).
17. 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).
18. Dejnirattisai, W. et al. Cross-reacting antibodies enhance dengue virus infection in humans. Science 328, 745-748, doi:10.1126/science.1185181 (2010).

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Project 16: Wilms Tumour 1 (WT1) specific T cells restricted by HLA-E

Primary supervisor: *Associate Prof. Geraldine Gillespie*

Co-supervisor: *Prof. Andrew McMichael*

Contact: geraldine.gillespie@ndm.ox.ac.uk

Project Overview

Background: WT1 is a developmental cell transcription factor that is over-expressed in a number of cancers, including acute myeloid leukaemia (AML). HLA-E is a non-polymorphic HLA class I molecule that is expressed at low levels in normal cells, but at higher levels in many cancers especially when classical HLA is downregulated. HLA-E normally binds a single peptide (VL9), derived from classical HLA class I leader sequence, and then binds the NKG2 receptors that regulate natural killer cells. Overexpression of HLA-E on cancer cells inhibits NK cell attack. We have shown that HLA-E can also present non-VL9 peptides to T cells, but these peptides have low affinity and only a minority stabilise HLA-E well. However, we have identified a peptide in WT1 that binds strongly to HLA-E and can prime human T cells in vitro. These T cells inhibit growth of AML cell line cells in vitro.

Approach: In this project we will generate additional T cell clones specific for this epitope using a new in vitro priming technique we have developed that generates higher affinity T cells. We will then transduce their T cell receptors into SKW3 and primary $CD8^+$ T cells and will test these T cells on AML cell lines in a growth inhibition assay and a cell lysis assay. We will also test these T cells on leukaemic cells obtained from bone marrow or patients in collaboration with Prof Paresh Vyas (WIMM).

We will compare recognition of WT1 peptide on AML cells by these T cells with recognition by a monoclonal antibody that have developed with Prof Xiaoning Xu at UCL. He has made a CAR-T cell from this antibody. Finally, we will compare the T cell clones and CAR -T for suppression of AML leukaemic cell growth in a NSGW mouse model, giving us an in vivo measurement of leukaemia suppression.

In parallel with the above we will identify additional epitopes in other proteins that are over-expressed in AML cells. For this we will use our established methods of epitope prediction, binding assays, structural analysis and stable tetramer preparation. These will be used to build up a multi-specific, polyclonal therapeutic T cell strategy.

Training Opportunities

The student will be trained in HLA-E biochemistry, protein preparation, T cell cloning, T cell receptor sequencing and transduction, T cell assays and suppression of growth of leukaemic cell in vitro, and also, the use of mouse models of human leukaemia. The latter studies will include training in animal experimentation under UK Home Office regulations. On completion of the project, the student should be capable of setting up similar studies for any cancer antigen.

Supervisor / Short Profile

Associate Professor Geraldine Gillespie.

Qualified in the biological sciences, with 30 years' experience in human T cell immunology and the biochemistry of MHC-T cell recognition. Key discoveries on T cell recognition in HIV and autoimmunity, MHC Ia plus HLA-E biochemistry, structures and T cell recognition. Has successfully trained both MSc and DPhil students within Oxford.

Emeritus Professor Andrew McMichael.

Clinically qualified scientist with 45 years' experience in human T cell immunology with major discoveries on T cell recognition of peptides, HLA-E interaction with NK cells and T cells, virus immune escapee, generation of monoclonal antibodies and vaccine development. Has trained successfully more than 80 DPhil students. Formerly he was Director of the MRC Human Immunology Unit and of the Weatherall Institute of Molecular Medicine. He has been awarded FRS, FMedSci, EMBO membership and a

Knighthood.

Key Publications (Gillespie and McMichael) :

1. Hansen, S. G. et al. Broadly targeted CD8(+) T cell responses restricted by major histocompatibility complex E. Science 351, 714-720, doi:10.1126/science.aac9475 (2016).
2. Li, D. et al. Mouse and human antibodies bind HLA-E-leader peptide complexes and enhance NK cell cytotoxicity. Commun Biol 5, 271, doi:10.1038/s42003-022-03183-5 (2022).
3. Walters, L. C. et al. Pathogen-derived HLA-E bound epitopes reveal broad primary anchor pocket tolerability and conformationally malleable peptide binding. Nat Commun 9, 3137, doi:10.1038/s41467-018-05459-z (2018).
4. Yang, H. et al. HLA-E-restricted, Gag-specific CD8(+) T cells can suppress HIV-1 infection, offering vaccine opportunities. Sci Immunol 6, doi:10.1126/sciimmunol.abg1703 (2021).
5. Walters, L. C. et al. Primary and secondary functions of HLA-E are determined by stability and conformation of the peptide-bound complexes. Cell Rep 39, 110959, doi:10.1016/j.celrep.2022.110959 (2022).

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Project 17: Genomic instability and oncogenic driver mutations underpin B cell-rich tertiary lymphoid structures associated with tumour-specific antibodies in colorectal cancer

Primary supervisor: *Prof Tim Elliott*

Secondary supervisor: *Dr Pedroza-Pacheco*

Contact: tim.elliott@ndm.ox.ac.uk

Project Overview

I Colorectal cancers (CRCs) are a significant worldwide public health concern with 1.93 million new cases and 0.94 million deaths reported in 2020, representing 10% of the global cancer incidence. From the clinical standpoint, CRCs are complex to diagnose and treat as they are generally asymptomatic and highly heterogeneous, often leading to the detection of advanced cancers. Over the last couple of years, a convincing body of evidence has emerged revealing the potential role of antibodies with diagnostic and/or therapeutic potential. High prevalence of endogenous antibodies that can directly bind tumour surfaces and the presence of B-cell-rich tertiary lymphoid structures (TLS) have been recently associated with overall survival and better response to immunotherapy in ovarian cancer[1,2]. In colorectal cancer (CRC), more nuanced results have been published. In early-stage patients, TLS were associated with better prognosis and lower risk of recurrence after resection, suggesting immune-derived benefit. Notably, TLS were predominantly detected in patients with genomic instability and higher prevalence of oncogenic driver mutations, increasingly known to underpin enhanced antigen availability, increased T cell infiltrates, and impact cellular processes in the tumour microenvironment (TME). Since antigen exposure and T cell help are critical in TLS formation, it is not surprising to find a higher incidence of TLS near the tumours of some patients. Yet, their interindividual variation in their cellular composition, spatial organisation, and the mechanisms regulating tumour-specific antibodies, remain unclear.

A possible molecular mechanistic explanation may lie in specific driver mutations underlying TLS. Mutations in the *BRAF* and *KRAS* genes are particularly interesting because of their prognostic value in CRC and their potential to modulate B cell receptor signalling via the extracellular signal-regulated kinase (ERK) pathway. Interestingly, small CRC cohort studies have shown that MSI and/or *BRAF-mutated* tumours frequently occur near active and mature TLS, potentially supporting plasma cell differentiation.

We hypothesise that TLS can be modulated a) directly by oncogenic mutations impacting cellular processes involved in B/T cell effector and regulatory function in the TME or b) indirectly by microsatellite instability (MSI) driving B cell and T cell neoepitopes.

This research programme aims to systematically quantify the functional relationship between tumour-intrinsic molecular processes, and the formation, cellular composition, and spatial distribution of TLS within the TME associated with tumour-reactive antibody development. To this end, we aim to collect clinical, tissue samples, and molecular data from CRC patients via the ORB Bank, the Translational Gastroenterology Unit, and the CRUK Oxford Centre at the University of Oxford. To dissect whether MSI and *BRAF/KRAS* mutations contribute to TLS function and the development of tumour-reactive antibodies, we will focus on a subset of patients with consensus molecular subtype (CMS) 1 and 3 - enriched for MSI and KRAS mutations. Samples will then be further classified into five demographically matched CRC groups: (1) *BRAF*-mut microsatellite stable (MSS), (2) *BRAF*-wt MSS, (3) *KRAS*-mut MSS, (4) *KRAS*-wt MSS, (5) *BRAF*-wt *KRAS*-wt MSI. Individuals suspected of CRC with no pathological diagnosis will be recruited to form a control group of demographically matched healthy individuals. We will harness our expertise in antigen presentation[3] and the cellular immune environment regulating humoral responses[4,5], by adapting our established pipelines that systematically assess genetic, protein, and cellular variation.

First, we will use an innovative multiplex immunofluorescent system (Vectra Polaris) to visualise and quantify tumour binding by endogenous IgG and IgA antibodies from fresh-frozen (when available) or formalin-fixed paraffin-embedded sections (FFPE). To determine whether oncogenic mutations predict tumour-reactive antibody development, we will apply well-established systems-based unbiased and

biased mathematical models previously used in Dr Pedroza's laboratory. To characterise the spatial distribution of the cellular environment that promotes TLS-associated increased plasmablasts and tumour-reactive antibodies, we seek to utilise the novel Nanostring CosMx Spatial molecular analyser at CAMS-COI that will enable us to image TLS and their interactions with tumours at the cellular and subcellular level.

Second, we will develop TLS-like organoids from ascites fluid of patients with MSI and/or /BRAF/KRAS mutations to assess their immediate impact on B cell and T cell signalling (spectral flow cytometry), gene expression (single-cell RNA-Seq), and function (leveraging well-established Tfh-B cell help co-cultures). BRAF/KRAS inhibitors and Crisp-CAS9 techniques may be used to determine if function can be restored. To gain a deeper view of the architectural structure of *ex vivo* isolated spheroids, we will collaborate with Prof Marco Fritzsche, the director of the Oxford-ZEISS Centre of Excellence and an expert in fast 3D Lattice Light Sheet Microscopy (LLSM).

Finally, we will determine if BRAF/KRAS-driven effects versus (or in addition to) MS status modulate the ability of B cells to present tumour antigens to T cells. To this end, we will generate patient-derived B cell lines with MSI and/or BRAF/KRAS mutations to explore the B cell immunopeptidome presented to T cells, critical for anti-tumour responses. This part of the project will be supported by cutting-edge mass spectrometry instrumentation (Bruker timsTOF Pro 2 LC-MS system) for capturing the *in vivo* repertoire of B cell MHC bound peptides.

This research programme will a) provide deeper mechanistic understanding of the drivers of TLS formation in CRC; b) clarify the immunological mechanisms that underpin the protective effect of TLS in cancer; and c) leverage B cells to develop novel immunotherapies and antibody-based cancer vaccines to recreate a microenvironment beneficial for TLS formation in refractive CRC tumours.

Training Opportunities

- Well-established DPhil programme with defined milestones, ample training opportunities between COI and the Oxford Centre for Immuno-Oncology, and access to university/department-wide seminars by world-leading scientists.
- 38+ colour high-dimensional spectral flow cytometry and 2D/3D in vitro functional assays.
- Novel technologies such as spatial transcriptomics, immunopeptidomics, and 3D microscopy.
- Highly collaborative environment with expertise ranging from molecular/cellular biology to computational biology, as well as several other collaboration opportunities within the University of Oxford and worldwide.

Supervisor / Short Profile

Short Profile Supervisory Team | Prof Tim Elliott FMedSci left the University of Oxford with a first in Biochemistry in 1983 and completed his PhD in cancer immunotherapy at the University of Southampton in 1986. He did his postdoctoral training at the Massachusetts Institute of Technology before returning to the University of Oxford to join the Institute for Molecular Medicine in 1990. In 2000, he became Professor of Experimental Oncology and Director of the Centre for Cancer Immunology at the University of Southampton. In 2020 he was appointed to the Kidani Chair of Immuno-Oncology at the University of Oxford. Prof Elliott is a world leader in the field of antigen presentation and T cell biology and has incorporated discoveries in the areas of antigen processing, T cell regulation and immunodominance into the development of new cancer immunotherapies.

Dr Pedroza-Pacheco is a Career Development Fellow in the Nuffield Department of Medicine at the University of Oxford. She obtained her bioengineering and immunology training at the Monterrey Institute of Technology (ITESM, Mexico) and University College London (UCL, UK). She did her postdoctoral training at the University of Oxford, and in 2014 she joined the Consortium for HIV/AIDS Vaccine Development (CHAVD) as a young faculty member. Dr Pedroza-Pacheco's dual expertise in engineering and immunology gives her a unique skill set, enabling her to combine a systematic approach to understanding complex diseases in an integrative context. By developing techniques and technologies that comprehensively assess genetic and protein variations that shape cellular function, Dr Pedroza has made significant contributions in the field of HIV and vaccinology, from revealing cellular mechanisms that regulate Tfh and B cell responses to uncovering predictive cellular features needed in vaccination

strategies to develop bnAbs.

Key Publications

1. Fridman, W.H., Meylan, M., Petitprez, F., Sun, C.-M., Italiano, A., and Sautès-Fridman, C. (2022). B cells and tertiary lymphoid structures as determinants of tumour immune contexture and clinical outcome. Nat. Rev. Clin. Oncol.
2. Mazor, R.D., Nathan, N., Gilboa, A., Stoler-Barak, L., Moss, L., Solomonov, I., Hanuna, A., Divinsky, Y., Shmueli, M.D., Hezroni, H., et al. (2022). Tumor-reactive antibodies evolve from non-binding and autoreactive precursors. Cell.
3. Sugiyarto, G., Prossor, D., Dadas, O., Arcia-Anaya, E.D., **Elliott, T.**, and James, E. (2021). Protective low-avidity anti-tumour CD8+ T cells are selectively attenuated by regulatory T cells. Immunotherapy Advances.
4. Moody, M.A*, **Pedroza-Pacheco, I***, Vandergrift, N.A., Chui, C., Lloyd, K.E., Parks, R., Soderberg, K.A., Ogbe, A.T., Cohen, M.S., and Liao, H.-X. (2016). Immune perturbations in HIV–1-infected individuals who make broadly reactive neutralizing antibodies. Science immunology. (***co-first author**).
5. Bradley, T*, Peppa, D*, **Pedroza-Pacheco, I***, Li, D*, Cain, D.W., Henao, R., Venkat, V., Hora, B., Chen, Y., Vandergrift, N.A., et al. (2018). RAB11FIP5 Expression and Altered Natural Killer Cell Function Are Associated with Induction of HIV Broadly Neutralizing Antibody Responses. Cell. (***co-first author**).

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Project 18: Deciphering the role of glycocalyx remodeling enzymes in human T-cell immunity.

Primary supervisor: *Dr Pablo Cespedes*

Secondary supervisor: *Prof. Michael L. Dustin*

Contact: pablo.cespedes@kennedy.ox.ac.uk

Project Overview

The glycocalyx is the outermost layer of the cell formed by glycosylated membrane-anchored proteins, lipids, and RNAs, which collectively form a 'sugar canopy' on the cell surface[1,2]. Enzymes within this canopy expand the chemical diversity of embedded immune receptors by adding (a process termed glycosylation) or removing sugar moieties and modifying other functional groups on the amino acid side chains of newly synthesized proteins. In a stepwise process that starts in the endoplasmic reticulum, glycosyltransferases and glycosyl hydrolases decorate immune receptors with complex chains of monosaccharides, also known as glycans. These glycans support several cellular functions, including receptor quality control, cell adhesion, pathogen recognition, detoxification, trans-cellular signaling and development[3-5]. In T cells, key effector molecules and immune receptors are transferred as part of highly glycosylated extracellular particles6-8, and several lymphocyte activation and subset markers, such as CD57[9,10], CDw75/HB-6[11], CD77[12], and GL7[13] are glycoantigens with unknown molecular acceptors and immune functions. We hypothesize that these glycans (and their biosynthetic enzymes) modulate T cell homeostasis, signaling and effector function.

Most critically, glycosylations are species-specific, and non-human primates and rodents fail to recapitulate sugar decorations found in humans[13-15]. Therefore, understanding if and how glycosylation of immune receptors in T cells, cancer cells and infected cells affects the development of cellular immunity requires human-specific methods and currently nonexistent humanized animal models. Our team will use cutting-edge OMICs, molecular genetics, and imaging technologies to identify differentially expressed glycosyltransferases critical for T-cell function. We will develop methods to study T cell trafficking/migration, the formation of cell-cell contacts and the delivery of feed-forward signals, including cytokines and extracellular particles, from T cells to antigen-presenting cells (APCs). We will study both fundamental and translational aspects of T cell glycobiology and develop a novel molecular and cellular framework for studying the role of enzymes and PTMs in lymphocyte biology.

First, we will define the overarching hierarchy of enzymes modulating the assembly of specialized T cell junctions termed immunological synapses (ISs)[16]. ISs are dynamic molecular hubs formed at the interface of T cells and APCs engaged in physical contact, supporting the sensing of cognate antigens and accessory signals, thus ensuring a highly specific transfer of information. We will explore how differentially expressed (DE) glycosyltransferases and their substrate ligands regulate the helping, suppressor or killing activities of helper, regulatory and cytotoxic T cells, respectively. The candidate will learn and apply cutting-edge methods to study T cell-APC interactions at different length and time scales, from model membrane systems (minutes to hours) to lymphoid organoids (days to weeks). To ensure success, the candidate will also enjoy the co-supervision of Professor Michael L. Dustin, who pioneered the development of supported lipid bilayers and the study of T-cell immune synapses. Students will also have access to the Oxford-ZEISS Centre of Excellence in Biomedical Imaging located at the Old Road campus.

Since glycosyltransferases and glycosidases have the potential to influence receptor charge, diffusion, aggregation, 2D supramolecular segregation, stability, turnover and recycling, we will also investigate how enzyme gain and loss of function leads to altered immune receptor function in disease states, including infection and cancer[17]. To increase our project's scientific and medical impact, we will take our *in vitro* observations and study key enzymes, substrate immune receptors and related sugar-sensing checkpoint pathways in histopathological sections of human tissues and organoids and bespoke animal models. We will harness this knowledge to develop novel methods to study cell-cell communication, discover new biology and illuminate the development of therapeutics harnessing the glycobiology of human immunity[18]. Our long-term goal is to establish multiple intramural and international

collaborations to develop the next generation of human- specific *ex vivo* systems and humanized animal models mimicking closely immunity-relevant glycosylations and other PTMs.

Training Opportunities

Candidates wanting to join our team will be encouraged to engage in educational and collaborative opportunities within the CAMS OI and abroad. Opportunities include participation in conferences and workshops in relevant areas, including super-resolution microscopy, extracellular particle biology, immunology, cellular biology, and bioinformatics. The candidate will investigate whether and how a differentially expressed enzyme (or combination of enzymes) mechanistically regulates immune receptor trafficking, receptor-ligand interactions, and the effector output of human primary T cells (i.e., exerting activation, cytotoxicity, or suppression). As part of these efforts, the candidate will learn and have access to cutting-edge OMICs technologies such as spatial transcriptomics, metabolomics, and proteomics, alongside CRISPR-Cas9 genome editing, spectral and nanoflow cytometry, lattice-light sheet, light sheet, Airyscan confocal and TIRF/TIRF-SI microscopy to interrogate the physical interactions between T cells and APCs and at different spatiotemporal scales.

Supervisor / Short Profile

Dr Pablo Cespedes. The candidate will work with group leader Dr Pablo Cespedes in studying enzymes modulating the physical communication between T cells and APCs. Dr Pablo Cespedes holds a PhD in Biology, Molecular Genetics and Microbiology (Pontificia Universidad Católica de Chile) and dedicated his early career to studying the molecular mechanism of viral pathogenesis. After moving to the UK, Dr Cespedes undertook molecular and cellular immunology training at Professor Dustin's laboratory. As part of his efforts, Dr Cespedes developed bead-supported lipid bilayers (BSLBs) as a synthetic APC platform for the multifaceted study of receptor-ligand interactions and the molecular output of T cell synapses. BSLBs also provide an effective platform for the high-throughput screening and scoring of agents modulating T-cell synapses, including proteins, genes, drugs, and other cell regulators.

Professor Michael L. Dustin. Prof. Michael L. Dustin will be a co- supervisor. Professor Dustin holds a PhD in Cell and Developmental Biology (Harvard University). Professor Dustin pioneered the development of planar-supported lipid bilayers to study the molecular and cellular anatomy of cell-cell contacts formed by antigen-stimulated T cells, which Professor Dustin named immunological synapses. Dr Cespedes and Professor Michael L. Dustin have ample molecular and cellular immunology expertise.

Key Publications

1. Cespedes, P. F. et al. T-cell trans-synaptic vesicles are distinct and carry greater effector content than constitutive extracellular vesicles. Nat Commun 13, 3460 (2022).
2. Saliba, D. G. et al. Composition and structure of synaptic ectosomes exporting antigen receptor linked to functional CD40 ligand from helper T cells. Elife 8 (2019). https://doi.org:10.7554/eLife.47528.
3. Cespedes, P. F., Beckers, D., Dustin, M. L. & Sezgin, E. Model membrane systems to reconstitute immune cell signaling. FEBS J 288, 1070-1090 (2021). https://doi.org:10.1111/febs.15488.
4. Balint, S. et al. Supramolecular attack particles are autonomous killing entities released from cytotoxic T cells. Science 368, 897-901 (2020). https://doi.org:10.1126/science.aay9207.
5. Grakoui, A. et al. The immunological synapse: a molecular machine controlling T cell activation. Science 285, 221-227 (1999). https://doi.org:10.1126/science.285.5425.221.

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7. Cespedes, P. F. et al. T-cell trans-synaptic vesicles are distinct and carry greater effector content than constitutive extracellular vesicles. Nat Commun 13, 3460 (2022). https://doi.org:10.1038/s41467-022-31160- 3

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Project 19: Studying the cell-intrinsic and exogenous roles of peptidyl arginine deiminase 4 in T-cell immunity.

Primary supervisor: *Dr Pablo Cespedes*

Secondary supervisor: *Prof. Michael L. Dustin*

Contact: pablo.cespedes@kennedy.ox.ac.uk

Project Overview

Background:

Peptidyl arginine residues are important sites for protein regulation and the control of diverse cellular processes, including epigenetic regulation, protein-sugar and protein-nucleic acid interactions, and the regulation of cellular energetics[1,2]. The modification of peptidyl arginine by post-translational modification (PTM) enzymes is essential in regulating arginine-dependent cellular processes. Peptidyl arginine deiminases (PADs) are a family of Ca^{2+}-dependent enzymes catalyzing the conversion of positively charged peptidyl arginine residues into neutral peptidyl citrulline[3]. Following the shift in overall protein charge, citrullinated proteins change their interactome and enable different molecular processes to unfold, including chromatin remodelling via loss of DNA-histone interactions and the promotion of gene transcription through the prevention of histone arginine methylation. Furthermore, citrullination has been regarded as a key PTM in inflammation, immunity, and cancer. For instance, neutrophil extracellular trap (NET) release[2,4,5] and neutrophil-endothelial adhesion require the citrullination of endogenous histones and PDIA1[6], respectively. Also, the citrullination of extracellular fibronectin promotes focal adhesions and fibroblast migration via integrin $\alpha5\beta1$[7]. Boosted by inflammation and neutrophil NETosis, citrullination is also pivotal in producing neoantigens and the loss of tolerance in rheumatoid arthritis[8].

Five PADs have been described in humans (PADs 1-4 and 6), each with tissue-specific expression patterns and protein substrates. Among these, PAD4 is highly expressed in primary and secondary lymphoid tissues and is essential for the inflammatory and bactericidal response of neutrophils[2,9]. In humans, the functional significance of PAD4 in T cells remains poorly understood despite reports of strong expression in the thymus, the bone marrow, the spleen, and in $CD3^+$ cells infiltrating the synovium of rheumatoid arthritis patients[10,11]. We have recently found that PAD4 is expressed in human follicular helper T cells (TFH), regulatory T cells and cytotoxic T cells. Among others, we found that knocking down PAD4 in antigen-stimulated T cells leads to an impairment in the release of effector particles containing CD40L, a pivotal B cell activating factor. Others have found that Padi4-/- mice have a reduced severity of autoimmune arthritis[12] and a reduced autoantibody response and tissue damage in Systemic Lupus[13], suggesting a potential role of PAD4 in T-cell dependent humoral immune responses.

Approach:

We seek to unravel the pathways controlling PAD4 expression in T cells and the mechanisms by which T-cell intrinsic PAD4 regulates the release of effector molecules, including CD40L and B- cell aiding cytokines (IL-4, IL-21). We will also evaluate whether the enzyme regulates other T- cell functions, including cytotoxicity and regulation. Gain- and loss-of-function experiments using lymphoid organoids and genetically engineered mouse models will help us elucidate whether and how PAD4 regulates the physical communication between T cells and various antigen-presenting cells (APCs). More specifically, we will study how PAD4 deficiency shapes the secretome of different T cell populations and the supramolecular organization of their specific receptors and effectors within immunological synapses (ISs)[14]. ISs are specialized cellular junctions facilitating the sensing of cognate antigens and accessory signals in the cell-cell interface, thus ensuring a highly specific transfer of information between the T cell and its partner.

Our team will use cutting-edge OMICs, molecular genetics, and imaging technologies to evaluate whether and how PAD4 regulate global T-cell homeostasis, trafficking, activation and effector function. We will develop a novel molecular and cellular framework to study T cell trafficking/migration, the formation of

cell-cell contacts and the bidirectional exchange of feed- forward signals, including cytokines and extracellular particles, between T cells and APCs. The candidate will learn and apply cutting-edge methods to study T cell-APC interactions at different lengths and time scales, from model membrane systems (minutes to hours) to lymphoid organoids (days to weeks). To provide complementary experience, the candidate will also enjoy the co- supervision of Professor Michael L. Dustin, who pioneered the development of supported lipid bilayers and the study of T-cell immune synapses. Students will also have access to the Oxford- ZEISS Centre of Excellence in Biomedical Imaging located at the Old Road campus.

Recent findings suggest that expression of PAD4 in tumours promotes survival, migration, and metastasis of cancer cells across different models[15,16]. However, the molecular mechanisms supporting malignancy are elusive. We have recently observed that key cytokines and immune receptors such as interleukins, PD-L1, CD40L and Siglecs are citrullinated in conditions mimicking the exposure of these proteins to extracellular PAD4. Since citrullination has the potential to influence receptor charge, diffusion, aggregation, 2D supramolecular segregation, stability, turnover and recycling, we will also investigate whether and how extracellular PAD4 lead to altered receptor-ligand interactions in cancer. We hypothesize that the citrullination of extracellular matrix and cell surface components interfere with receptor-ligand interactions and the assembly of antitumoral synapses. To increase our project's scientific and medical impact, we will develop synthetic systems for studying the role of extracellular PADs and citrullination in receptor-ligand interactions required for cellular immunity, including the use of lymphoid and cancer organoids and bespoke animal models to study if and how extracellular PAD4 impairs pathogen and tumour clearance.

Training Opportunities

Candidates wanting to join our team will be encouraged to engage in educational and collaborative opportunities within the CAMS OI and abroad. Opportunities include participation in conferences and workshops in super-resolution microscopy, extracellular particle biology, immunology, cellular biology, and bioinformatics. The candidate will investigate whether and how a PAD4 mechanistically regulates immune receptor trafficking, receptor-ligand interactions, and the effector output of human primary T cells (i.e., exerting activation, cytotoxicity, or suppression). As part of these efforts, the candidate will learn and have access to cutting-edge OMICs technologies such as spatial transcriptomics, metabolomics, and proteomics, alongside CRISPR- Cas9 genome editing, spectral and nanoflow cytometry, lattice-light sheet, light sheet, Airyscan confocal and TIRF/TIRF-SI microscopy to interrogate the physical interactions between T cells and APCs and at different spatiotemporal scales.

Supervisor / Short profile

Dr Pablo Cespedes. The candidate will work with group leader Dr Pablo Cespedes in studying enzymes modulating the physical communication between T cells and APCs. Dr Pablo Cespedes holds a PhD in Biology, Molecular Genetics and Microbiology (Pontificia Universidad Católica de Chile) and dedicated his early career to studying the molecular mechanism of viral pathogenesis. After moving to the UK, Dr Cespedes undertook molecular and cellular immunology training at Professor Dustin's laboratory. As part of his efforts, Dr Cespedes developed bead-supported lipid bilayers (BSLBs) as a synthetic APC platform for the multifaceted study of receptor-ligand interactions and the molecular output of T-cell synapses. BSLBs also provide an effective platform for the high-throughput screening and scoring of agents modulating T-cell synapses, including proteins, genes, drugs, and other cell regulators.

Professor Michael L. Dustin. Professor Michael L. Dustin will be a co- supervisor. Professor Dustin holds a PhD in Cell and Developmental Biology (Harvard University). Professor Dustin pioneered the development of planar-supported lipid bilayers to study the molecular and cellular anatomy of cell-cell contacts formed by antigen-stimulated T cells, which Professor Dustin named immunological synapses. Dr Cespedes and Professor Michael L. Dustin have ample molecular and cellular immunology expertise.

Key Publications

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Project 20: How interactions between HIV and Dendritic Cells can translate into distinct pathways of antigen delivery and presentation: implications for precision vaccine development

Primary supervisor: *Prof. Sarah Rowland-Jones*

Secondary supervisor: *Dr Ester Gea-Mallorquí*

Contact: *sarah.rowland-jones@ndm.ox.ac.uk*

Project Overview

This project will make use of marked differences that we have observed in the way two closely related human retroviruses interact with dendritic cells to develop strategies that optimize the generation of potent T-cell responses against immunogens from persistent viruses and tumours.

HIV-1 is responsible for the global AIDS pandemic, estimated to have caused 40 million deaths. HIV-2 is a genetically and structurally similar virus that can also cause AIDS. However, HIV-2 is more readily controlled by the immune system, and is frequently associated with long-term non-progression, even without treatment.

Our group aims to understand mechanisms of viral control by investigating the biology of HIV-2 infection. We have previously shown that HIV-2 viral control correlates with the potent induction of polyfunctional and high avidity cytotoxic T lymphocytes (CTLs)[1–3]. The generation of similarly potent polyfunctional T-cell responses against tumour antigens is a key goal for therapeutic cancer vacines. In this project we plan to investigate the initial priming of the adaptive response by HIV-2, which is a critical step for induction of a strong CD8+ T-cell response.

Dendritic cells (DCs) play a pivotal role in priming adaptive immune responses. As professional antigen presenting cells, DCs instruct the development of the adaptive response, mainly through antigen presentation. How an antigen is delivered to DCs can have a profound impact on its processing, loading into the antigen presenting pathway and subsequent presentation to the adaptive immune system.

Typically, intracellular antigens will be processed by the proteasome and loaded into major histocompatibility complex (MHC) class I molecules; while internalised external antigens will be digested and loaded into MHC class II in late endosomes before being transported to the plasma membrane. However, external antigens can also be presented via MHCI in a process known as cross-presentation, which has been shown to be particularly powerful in inducing potent CD8+ T cell responses.

Pathogenic HIV-1 and immune-controlled HIV-2 interact differently with DCs[4–5]. Our initial observations show that HIV-1 is more likely to be captured on the surface of DCs, whereas very little HIV-2 is captured so the virus largely infects DCs. We therefore hypothesise that the different interactions between HIV-1 and HIV-2 with DCs translate into distinct pathways of antigen delivery, presentation and further development of the adaptive response, and will ultimately correlate with long-term viral control. In this project we aim to identify and characterize the viral proteins responsible for these distinct interactions with DCs, and then investigate how these proteins can be utilized in vaccine constructs to dictate the nature of the T-cell response induced by the vaccine.

Training Opportunities

This project offers the candidate an excellent opportunity for training in a multi-disciplinary range of skills and state-of-the-art laboratory techniques, including cellular immunology (flow cytometry, dendritic cell culture and functional assays of T-cell activity), virology, proteomics analysis, confocal microscopy and antigen processing studies. In addition, analysis of laboratory data in relation clinical data will be performed to understand the potential translational impact of the laboratory studies.

Additional training on scientific writing and presentation of research findings will be available to the student: he/she will be encouraged to prepare and present his/her work during regular weekly meetings/scientific presentations, take part in regular journal clubs, and attend national/international

conferences, as well as preparing the first draft of manuscripts and review articles in close collaboration with the supervisors.

Professor Rowland-Jones is an active clinician (in adult Infectious Diseases) and would be happy to support a medically-qualified candidate in obtaining clinical experience in Oxford to gain insights into medical practice in the UK.

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Project 21: Assessing the evidence for introducing cardiac screening in pregnancy: are the criteria for a screening programme fulfilled?

Primary supervisor: *Professor Marian Knight*

Secondary supervisor: *Dr Rema Ramakrishnan & Dr Joris Hemelaar*

Contact: marian.knight@npeu.ox.ac.uk

Project Overview

Cardiac disease has been the leading cause of death during pregnancy and in the immediate postnatal period for the past 20 years [1], and is the leading cause of maternal death in many other countries [2, 3]. As the characteristics of the population of women giving birth continues to change, with women choosing to give birth at older ages, and with a greater number of cardiovascular risk factors such as smoking, obesity and hypertension, it is likely that preventing maternal cardiac deaths will become even more important. However, 90% of women who die from cardiac diseases during pregnancy or in the postnatal year in the UK are not known to have cardiac disease before they become pregnant [1]. This raises the question of whether we should be screening for cardiac disease during pregnancy in order to prevent future morbidity or mortality. However, there are a number of gaps in the evidence which need to be filled to allow assessment of the potential utility of a national screening programme based on the UK National Screening Committee criteria [4] (modified from Wilson and Jungner, 1968 [5]). This project aims to fill those gaps using the following analyses:

- A systematic review to assess the burden of maternal cardiac disease morbidity and mortality in high resource settings, and interventions to assess and treat identified conditions.
- Analysis of UK linked datasets to assess the types and burden of cardiac disease morbidity diagnosed during or in the year after pregnancy, and their long-term outcomes to fully describe the natural history of each disorder.
- Assessment of the different screening tools available, their performance and costs.
- Preliminary economic evaluation of the proposed screening programme.
- Investigation of the acceptability of the proposed programme to women, clinicians and policy-makers

Training Opportunities

This project will involve detailed analysis and interpretation of existing data, as well as primary data collection and analysis. Primary data collection may be either qualitative or quantitative, depending on the candidate student's interest, and could include assessment of basic imaging methods. The student will work within a multi-disciplinary team and will gain research experience in literature review, epidemiological and statistical methodology, health economics, study design, data collection and analysis. NDPH students have access to relevant modules from the MSc in Global Health and Epidemiology to obtain formal training in relevant techniques. Regular research meetings and workshops will be held which the candidate will be expected to attend and to present research findings.

Supervisor / Short Profile

Professor Marian Knight is a Public Health Physician and applied health researcher in the National Perinatal Epidemiology Unit, Nuffield Department of Population Health whose research focuses on the care and prevention of severe complications of pregnancy and early life and addressing disparities in outcomes for women and babies from different population groups. She established the UK Obstetric Surveillance System (UKOSS) in 2005 to conduct national studies of severe morbidities in pregnancy and leads the MBRRACE-UK national Confidential Enquiries into Maternal Deaths and Morbidity. She leads a parallel stream of work to improve the care of babies who undergo surgery in early life. She was elected to fellowship of the Academy of Medical Sciences in 2021 in recognition of her exceptional contribution to the advancement of medical science, and awarded an MBE in the 2023 King's New Year Honours for

services to Maternal and Public Health.

Additional statistical supervision will be provided by Dr Rema Ramakrishnan and the health economics team at the National Perinatal Epidemiology Unit, led by A/Professor Oliver Rivero-Arias

Other Clinical Supervisors

Dr Joris Hemelaar, Consultant Obstetrician and Senior Clinical Research Fellow, National Perinatal Epidemiology Unit, Nuffield Department of Population Health and John Radcliffe Hospital, Oxford

Key Publications

1. Knight, M., et al., eds. Saving Lives, Improving Mothers' Care - Lessons learned to inform maternity care from the UK and Ireland Confidential Enquiries into Maternal Deaths and Morbidity 2018-20. 2022, National Perinatal Epidemiology Unit, University of Oxford: Oxford. https://www.npeu.ox.ac.uk/assets/downloads/mbrrace-uk/reports/maternal-report-2022/MBRRACE-UK_Maternal_MAIN_Report_2022_v10.pdf
2. Heemelaar, S., et al., Maternal mortality due to cardiac disease in low- and middle-income countries. Trop Med Int Health, 2020. 25(6): p. 673-686.
3. Regitz-Zagrosek, V., et al., 2018 ESC Guidelines for the management of cardiovascular diseases during pregnancy. Eur Heart J, 2018. 39(34): p. 3165-3241.
4. UK National Screening Committee. Criteria for a population screening programme. https://www.gov.uk/government/publications/evidence-review-criteria-national-screening-programmes/criteria-for-appraising-the-viability-effectiveness-and-appropriateness-of-a-screening-programme
5. Wilson JMG, Jungner G. Principles and practice of screening for disease. Public Health Paper Number 34. Geneva: WHO, 1968.

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Project 22: Modulating myeloid metabolism to moderate age-related chronic inflammation

Supervisor: *Assoc Prof James Fullerton (Clinical)*

Co-supervisors: *Dr Roel De Maeyer (Non-clinical)*

Contact: *james.fullerton@ndorms.ox.ac.uk*

Project Overview

Ageing is associated with the development of multiple chronic diseases and results in both increased susceptibility to infection and reduced vaccine efficacy. The unifying pathophysiology underlying these disparate effects appears to be a dysregulated immune system resulting in a low-grade chronic systemic inflammatory phenotype termed 'inflammageing'. Whilst the biological basis for this phenomenon is currently unknown, mononuclear phagocytes, cells central to the regulation of inflammation and immunity, have been identified as key contributors.

Persistent macrophage activity results in the secretion of pro-inflammatory cytokines, but when locked in this state, these cells paradoxically exhibit defective phagocytosis and efferocytosis. The resulting accumulation in cellular debris exacerbates inflammation and prevents resolution, triggering a 'vicious cycle'. Recent evidence suggests that the macrophages underlying this pathological phenotype are highly glycolytic and that this can be targeted pharmacologically, however the precise interaction between inflammation, phagocytosis and metabolism are poorly understood, particularly in humans.

This project consequently seeks to establish if modulating macrophage metabolism can moderate chronic inflammation in ageing.

To address this aim, we will utilise novel human immune challenge (HIC) models to investigate and compare the role of metabolism in the activation of monocytes and macrophages. We will use different stimuli injected into the skin of young (18-35 years) and older (60+ years) volunteers. Hallmarks of inflammation and its resolution will be assessed using novel non-invasive imaging techniques, while biopsies and blisters will provide insight into the immunometabolic environment in the skin. Key readouts will include macrophage phagocytosis and their interaction with T cells. Moreover, based on existing literature and our data, a detailed study of the potential of dimethyl fumarate, an immunomodulatory drug currently used clinically to treat patients with multiple sclerosis, to modulate monocyte/macrophage metabolism in vivo and in vitro will be undertaken. In addition to skin, we will investigate blood and obtain fine needle aspirates of draining lymph nodes to see how modulating metabolism alters inflammation locally at the site of injury as well as dictates downstream T cell-mediated immunity.

Training Opportunities

The successful applicant will benefit from regular hands-on training and supervision by experienced laboratory and clinical scientists at the COI (under Prof Tao Dong's tutelage), Botnar Research Centre and Kennedy Institute. As well as developing core 'wet lab' skills (flow cytometry, cell culture, metabolic assays etc) they will gain exposure to a range of cutting-edge techniques (including single-cell RNA sequencing) and analysis. This project will feature multiple assays focusing on macrophage function and how they interact with T cells to orchestrate inflammation and immunity. Most uniquely, they will work in the new NIHR Experimental Medicine Clinical Research Facility to undertake and obtain samples from the HIC paradigms; literally moving from 'bed to benchside'.

The development of outstanding communication and project management skills is expected as they take on the significant responsibility of establishing and running experimental medicine studies. To achieve this they will be supported, trained and mentored throughout. Daily interaction with fellow clinical and non-clinical PhD students and post-doctoral researchers will be supplemented by frequent supervisory meetings with Dr Fullerton and Dr De Maeyer. Regular attendance and participation at lab meetings, in Prof Tao Dong's group at the COI and those of the Oxford Centre for Clinical Therapeutics (led by Prof Duncan Richards) will be required. Presentation at international conferences and publication in leading

biomedical journals is expected. The quality, relevance and impact of the students work will be guaranteed by the inter-linked nature of this work with existing research programmes (e.g. A-TAP) and industrial collaborations (e.g. Bristol Myers Squibb), with associated expertise and funding. Students will also be required to attend regular seminars within the Department and those relevant in the wider University.

Students will have access to various courses run by the Medical Sciences Division Skills Training Team and other Departments. All students are required to attend a 2-day Statistical and Experimental Design course at NDORMS (Nuffield Department of Orthopaedics) and run by the IT department (information will be provided once accepted to the programme).

Supervisor / Short Profile

Dr Fullerton currently splits his time between the University, where he is Associate Professor of Clinical Therapeutics, and the John Radcliffe Hospital, where he is an Acute General Medicine Physician and Clinical Pharmacologist. In this latter role he contributes to both the Medicines Management and Therapeutics Committee and the Oxford University Hospitals/University of Oxford Advanced Therapy Medicinal Products / Genetic Modification Safety Committee. Externally he remains an Honorary Clinical Lecturer at UCL, sits on the Resuscitation Council (UK) Advanced Life Support Committee and is an Executive Editor for the British Journal of Clinical Pharmacology. He previously sat on UCL Research Ethics Committee for several years. Dr Fullerton's research focuses on the use of experimental medicine studies to promote scientific translation for patient benefit. In particular he is interested in the utility and development of human immune challenge models, seeking to design novel paradigms that will enable and catalyse the work of academic and industrial partners. Clinically, he is interested in understanding the host immune response to inflammatory stimuli, most notably infection, and how this can inform decision making in the context of acute, unscheduled care episodes. James sits on the Management Committee of the NIHR Oxford Experimental Medicine Clinical Research Facility, is Research Lead for Acute General Medicine at the John Radcliffe Hospital and co-ordinates the Academic Centre for UrgenT and Emergency Care (ACUTECare).

Dr De Maeyer is an early career researcher currently holding an Oxford-BMS fellowship focusing on establishing novel human immune challenge models to provide robust and reproducible platforms with which to interrogate early phase experimental medicines. Dr De Maeyer has an interest in understanding settings of dysregulated immunity such as ageing and autoimmunity using these HIC models. He has expertise in performing intricate functional assays that inform on cellular function, metabolism, and interaction using samples derived from HIC models, using these in combination with blood and omics approaches to gain deeper insight into human immune function.

Key Publications

1. De Maeyer et al. Blocking elevated p38 MAPK restores efferocytosis and inflammatory resolution in the elderly. Nat Immunol 2020. https://doi.org/10.1038/s41590-020-0646-0
2. Chambers et al. Recruitment of inflammatory monocytes by senescent fibroblasts inhibits antigen-specific tissue immunity during human aging. Nat Aging 2021. https://doi.org/10.1038/s43587-020-00010-6
3. Desdín-Micó et al. T cells with dysfunctional mitochondria induce multimorbidity and premature senescence. Science 2020. https://doi.org/10.1126/science.aax0860
4. Florian et al. Translational drug discovery and development with the use of tissue-relevant biomarkers: Towards more physiological relevance and better prediction of clinical efficacy. Exp Dermatol 2020. https://doi.org/10.1111/exd.13942
5. Drennan et al. In vivo human keyhole limpet hemocyanin challenge in early phase drug development: A systematic review. Clin Transl Sci 2022. https://doi.org/10.1111/cts.13457

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Project 23: Identification of host restriction factors for poxviruses and development of antivirals

Supervisor: *Professor Geoffrey L. Smith*

Contact: *geoffey.smith@path.ox.ac.uk*

Project Overview

Background

Poxviruses are complex viruses that replicate in the cell cytoplasm and have large dsDNA genomes. The most infamous poxvirus was variola virus (VARV), the cause of smallpox, a disease eradicated by widespread vaccination with the related orthopoxvirus, vaccinia virus (VACV). Following the elimination of smallpox in 1979, only a few poxviruses have caused disease in humans, these include molluscum contagiosum virus (MOCV), orf virus and the orthopoxviruses cowpox virus and monkeypox virus (MPXV). Human infections by orf virus, cowpox virus and MPXV have been zoonoses, with limited human-to-human transmission, until 2022, when a large epidemic of MPXV spread throughout the world and caused > 85,000 confirmed cases that were mostly reported in men who have sex with men. Fortunately, this epidemic was caused by clade II MPXV strains that originate in West Africa and which generally produce mild infections unless the patient is immunosuppressed due to, for instance, co-infection with human immunodeficiency virus (HIV). In contrast, the more dangerous clade I MPXV strains originate in Central African and induce mortality at about 8%. MOCV is a human specific poxvirus that causes benign dermal proliferations, often in children, that, although unsightly, usually do not cause serious disease unless the patient is immunodeficient. There are currently no drugs available to treat MOCV. Two drugs, tecovirimat and tembexa, were developed and licensed against smallpox and are also effective against other orthopoxviruses such as MPXV. However, recently the use of tecovirimat in MPXV-infected humans had led to emergence of drug resistant MPXV due to mutation in the virus protein target of tecovirimat, protein F13.

The project aims to identify host proteins that function as poxvirus restriction factors and develop novel drugs against poxviruses that target cellular proteins with proviral activity. By targetting a cellular protein, it is difficult for the virus to develop drug resistance by mutation.

Preliminary Data

A proteomic study of VACV-infected human fibroblasts revealed that of the ~ 9000 cellular proteins detected, 265 were down-regulated more than 2-fold and mostly in a proteasome-dependent manner. A hypothesis to explain this targeted degradation, was that the degraded proteins had antiviral functions (virus restriction factors) and that was why the virus had evolved a mechanism to degrade them. This hypothesis was later confirmed for histone deacetylase 4 and 5 (refs 1 & 2) and for tripartite motif protein 5α (TRIM5α) (ref 5). This project proposal derives from a study of TRIM5α, which is a well-known restriction factor for RNA viruses, such as HIV-1, but hitherto is less studied against DNA viruses. Our data show that TRIM5α also restricts orthopoxviruses and, via its SPRY domain, binds the orthopoxvirus capsid protein L3 to diminish virus replication and activate innate immunity. In response, several orthopoxviruses, including VACV, MPXV, VARV, deploy countermeasures. First, viral protein C6 binds directly to TRIM5 via the RING domain to induce its proteasome-dependent degradation. Second, cyclophilin A (CypA) is recruited via interaction with protein L3 to virus factories and virions to antagonise TRIM5α, and this interaction is prevented by cyclosporine A (CsA) and non-immunosuppressive derivatives alisporivir and NIM811. CsA, alisporivir and NIM811 have antiviral activity against orthopoxviruses including MPXV and since these drugs target a cellular protein, CypA, emergence of virus drug resistance is difficult. These results warrant testing of CsA derivatives against orthopoxviruses including MPXV and VARV. An initial collaboration with the CDC, USA has been established to do this. A paper describing these findings has been reviewed very favourably at Nature and is being revised.

Experimental Proposal

Capsid protein L3. The VACV capsid protein L3, which is bound by both the antiviral protein TRIM5α and the proviral protein CypA, is highly conserved in sequence and function amongst the orthopoxviruses, but its conservation extends beyond the orthopoxviruses to all chordopoxviruses, including MOCV and poxviruses of veterinary importance and zoonotic threat to humans (such as the capripoxviruses and parapoxviruses, for instance). Therefore, the L3 orthologues from these different chordopoxviruses will be tested for their ability to bind human TRIM5α and CypA, or the corresponding orthologues from the natural host species of these viruses. If interactions are found, then CsA or non-immunosuppressive derivatives will be tested against these viruses in cell culture and animal models. MOCV cannot be grown in cell culture and so we will establish a collaboration with a clinical dermatologist to evaluate if these compounds have clinical benefit in treatment of established MOCV infections.

To aid drug development, and to obtain a greater mechanistic understanding of how TRIM5α mediates antiviral activity, and how CypA antagonises this, the project will study in greater detail the C6:TRIM5α, L3:TRIM5α, and L3:CypA interactions by structural biology and then target these protein interfaces to develop additional antiviral compounds. Collaborations between ourselves and structural biology labs in Oxford (Dave Stuart) and Beijing (George Gao) have been initiated.

Training Opportunities

The student appointed to this multidisciplinary project will have multiple training opportunities in UK and China. He / she will:

- undertake molecular characterisation of viral proteins and how these interact with and exploit or antagonise cellular factors
- be trained in classical virology techniques such as virus growth, purification, titration and the design and construction of recombinant viruses
- learn methodology to measure activation of innate immunity, such as NF-κB, and how this is antagonised by poxvirus proteins
- in collaboration with structural biology labs learn methods for protein production and purification, and protein structure determination by X-ray crystallography or cryoEM
- determine if existing drugs can be re-purposed against poxviruses, or whether better drugs can be developed based on knowledge of protein-protein interfaces
- interact with clinicians to determine if such compounds have clinical benefit against human infections with poxviruses such as MOCV and MPXV

Supervisor / Short Profile

Geoffrey L. Smith obtained his PhD (1981) for work with influenza virus in Alan Hay's laboratory at the National Institute for Medical Research, London. Then as a postdoc in Bernard Moss's laboratory at the National Institutes of Health, USA (1981-84), together with Michael Mackett, he developed vaccinia virus (the smallpox vaccine) as an expression vector and established the principal of using genetically engineered viruses as live vaccines. He continued studying poxviruses after returning to UK at Cambridge (1985-9), Oxford (1989-2000), Imperial College London (2000-11), Cambridge (2011-22) and again at Oxford from 2023. His research studies the interactions of poxviruses with the host cell and immune system. Forty-six PhD / DPhil students have graduated from his laboratory.

He has had several roles promoting microbiology and advising on science policy. These include having been Chairman of the WHO Advisory Committee for Variola Virus (smallpox) Research, Chairman of the Scientific Advisory Board of the Centre for Structural and Systems Biology Hamburg, a member of the University Research Grants Council Hong Kong, President of the International Union of Microbiological Societies, Chairman of the Royal Society Committee for Scientific Aspects of International Security, Chairman of the Scientific Advisory Board of the Friedrich-Loeffler Institute (German Ministry for Food and Agriculture), a member of the Royal Society Science Policy Advisory Group, a Governor of the Lister Institute of Preventive Medicine and Chairman of the Scientific Advisory Board of the Pirbright Institute. He was elected a Fellow of the Academy of Medical Sciences (2002), the Institute of Biology (2002), the Royal Society (2003), a Founding Member of the European Academy of Microbiology (2008) and a member of Leopoldina - the German National Academy of Sciences (2011). In 2005 he was awarded the

Feldberg Foundation Prize in Medical and Biological Science, in 2012 the GlaxoSmithKline / American Society for Microbiology International Member of the Year Award and in 2016 the Loeffler-Lecture, which is a honorary lecture jointly organized by the Friedrich-Loeffler-Institut and the Alfried Krupp Wissenschaftskolleg (scientific college) in Greifswald, Germany. He was the recipient of the Marjory Stephenson Prize Lecture from the Microbiology Society (UK) in 2018 and the Leeuwenhoek Medal and Prize Lecture from the Royal Society 2019.

Clinical Supervisor(s)

A clinical supervisor of this project has not yet been appointed, but we would be delighted to make such an appointment either following advice from COI, or by approaching clinical dermatologists or virologists to test candidate drugs against poxvirus infections of humans.

Key Publications

1. Soday, L., Lu, Y., Albarnaz, J.D., Davies, C., Antrobus, R., Smith, G.L., & Weekes, M.P. (2019). Quantitative temporal viromics of vaccinia virus infection reveals regulation of histone deacetylases by a virus interferon antagonist. Cell Reports 27, 1920-33 e7.

2. Lu, Y., Stuart, J.H., Talbot-Cooper, C., Agrawal-Singh, SA., Huntly, B., Smid, A.I., Snowden, J.S., Dupont, L., & Smith, G.L. (2019) Histone deacetylase 4 promotes type I interferon signalling, restricts DNA viruses, and is degraded by vaccinia virus protein C6. Proc. Natl. Acad. Sci. USA 116, 11997-12006.

3. Albarnaz, J.D., Ren, H., Torres, A.A., Shmeleva, E.V., de Melo, C.M.A.G., Bannister, A.J. & Smith, G.L. (2022). Molecular mimicry of NF-kappaB by vaccinia virus protein enables selective inhibition of antiviral responses. Nature Microbiol. 7, 154-68.

4. Talbot-Cooper, C., Pantelejevs, T., Shannon, J.P., Cherry, C.R., Au, M.T., Hyvönen, M., Hickman, H.D. & Smith, G.L. (2022). Poxviruses and paramyxoviruses use a conserved mechanism of STAT1 antagonism to inhibit interferon signaling. Cell Host Microbe 30, 1-16

5. Zhao, Y., Lu, Y., Richardson, S, Sreekumar, M., Albarnaz, J.D. & Smith, G.L. (2023) TRIM5α restricts poxviruses, viral evasion and drugs for mpox and smallpox. Nature, under revision.

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