Project 1-1

Project Title: T cell immunity in COVID19 infection – Understanding the contribution of antigen specific T cells to SARS-CoV-2 viral control and clinical outcomes.

Supervisor: Professor Ellie Barnes - ellie.barnes@ndm.ox.ac.uk

Project Overview

This project is a fantastic opportunity to join the team at Oxford University studying the role of T cell immunity in COVID-19. In the last 6 months the project supervisors have developed local and national programs in COVID 19 in order to assess the contribution of T cell immunity to viral control and pathogenesis.

Cohorts available for analysis include:

1. > 300 health care workers recruited at local hospitals during primary infection and followed longitudinally over time.

2. A national cohort of >2,000 health care workers recruited and followed prospectively (approximately 50% SARS-COV2 infected and 50% naïve at recruitment).

The successful applicant will use these cohorts to address the following fundamental questions:

1. What are the T cell responses acquired following SARS-CoV-2 infection and do these protect against future infections?

This will be an opportunity to understand antigen specific T cell specificity, function, phenotype, and magnitude, stratified by clinical characteristics, before and after SARS-CoV-2 infection using advanced immune techniques (as described below – training opportunities)

2. Do cross-reactive T cell responses from seasonal coronavirus exposure contribute to protection?

The role of prior exposure to human seasonal coronaviruses including alpha coronaviruses (HCoV-NL63 and HCoV-229E), and beta coronaviruses (HCoV-HKU1 and HCoV-OC43), that may generate SARS-CoV-2 cross-reactive T cell immune responses, is of enormous interest and will be evaluated using viral specific peptides in immune assays.

SARS-CoV-2 and seasonal coronavirus specific T cells may be further defined through the detailed analysis of the RNA transcriptome of viral specific T cells using HLA class-I and II pentamers, and evaluated in in vitro killing assays.

3. What is the duration of T cell immunity (assessed alongside humoral immune responses with collaborators)?

COVID-19 Immunology at the Oxford University operates in a highly effective, stimulating, interdisciplinary environment. Working closely with others, the applicant will track the fate of SARS-CoV2 specific T cells alongside humoral responses to SARS-CoV02 and other seasonal coronaviruses in the prospective cohort study of healthcare workers.

Training opportunities

The student's PhD will give superb training in translational and basic immunology, integrating state-of the-art and established immune techniques (multi-parametric flow cytometric assays, T-cell ELISpot, proliferation assays, Intracellular cytokine assays and design/application of HLA-class I and II pentamers). Advanced single cell (sc)-RNA sequencing genomic and bioinformatic analysis may also be used in tetramer sorted T cell populations. T cell killing assays for COVID19 and seasonal coronoviruses cross-reactive T cells may be developed.

This studentship will be based at the Peter Medawar Building for Pathogen Research (PMB) equipped with cutting-edge facilities, Cat-3 laboratories with in-vitro cell culture systems of SARS-CoV-2 already established, and dedicated laboratory space for vaccine development. The PMB houses around 150 scientists working on HIV, HCV, influenza, TB, malaria, melioidosis and dengue. Academic excellence at the PMB is reflected in recent major papers in Nature, Science and the NEJM. The Infection and Immunity research element is the strongest in the Medical Sciences Division at Oxford. Visiting speakers of international repute host lectures here regularly.

Supervisors

Prof. Eleanor Barnes has a long-standing interest in viral pathogenesis, immunology and vaccine development. She leads a research group with a focus on T cell immunity and viral control, in association with viral genomic analysis. She has previously led the laboratory work into human experimental medicine studies with the aims of developing HCV and HBV simian adenoviral vectored vaccines for HBV immunotherapy and HCV prophylaxis. Recently she has been working closely with Susanna Dunachie and Paul Klenerman to establishing a major national program of work in T cell immunity in COVID-19. She has published >180 primary research peer-reviewed journal articles (with additional chapters and reviews) primarily in the field of hepatitis, vaccinology, hepatology and most recently COVID-19. Her research is consistently published in the leading specialist journals, most recently in Science Translational Medicine, Hepatology, and Nature Genetics (all as lead and/or corresponding author).

Prof. Susanna Dunachie is an infectious diseases clinician and Associate Professor in Tropical Medicine at the University of Oxford, undertaking research to understand the human immune response to COVID-19 and to neglected tropical diseases including scrub typhus and melioidosis. She spent four years living in Thailand and has ongoing research collaborations in Thailand, India and Bangladesh. In Oxford her research laboratory focuses on understanding why people with diabetes get more severe infections. She was recently awarded a Hamied Foundation / Academy of Medical Sciences Visiting Professorship to India, and is an Honorary Consultant in Tropical Medicine and Infectious Diseases at Oxford University Hospitals NHS Foundation Trust, where she is Travel Health Lead and runs the University's travel clinic.

Prof Paul Klenerman is overseeing immune assessment of COVID19 at University of Oxford. His main contribution to science include understanding mechanisms of viral persistence and control, including T cell escape and antagonism, and defining the key features of successful immune responses against HCV, leading to trials of a T cell vaccine; and to define the distinctive CD161+ T cell population, which dominates in the human liver.

Clinical Supervisor
All the PIs are also clinicians integrated within the clinical departments at Oxford University NHS Foundation Trust ensuring maximum clinical relevance of the project will be sought and disseminated.

Key publications

1. COVID-19 paper to be submitted to Nature Immunology and on BioRXiV..(add paper)
   Characterisation of the SARS-CoV-2 T cell immune response in PCR-confirmed SARS-CoV-2 infected subjects and seronegative subjects without known SARS CoV-2 exposure using a range of T cell assays that differentially capture immune cell function. We show that the detection of cross-reactive T cell responses to SARS-CoV-2 is critically dependent on the choice of assay used, and analysis of memory responses to specific non-spike proteins provides a way to distinguish recent infection from pre-existing immunity.

2. Esposito I, Cicconi P, D'Alise AM, Brown A, Esposito M, Swadling L, Holst PJ, Bassi MR, Stornaiuolo M, Mori F, Vassilev V, Li W, Donnison T, Gentile C, Turner B, von Delft A, Del Sorbo M, Barra F, Contino AM, Abbate A, Novellino E, Thomsen AR, Christensen JP, Lahm A, Grazioli F, Ammendola V, Siani L, Colloca S, Klenerman P, Nicosia A, Dorrell L, Folgori A, Capone S, **Barnes E**, on behalf of the PEACHI Consortium. MHC class II invariant chain–adjuvanted viral vectored vaccines enhances T cell responses in humans (lead and corresponding authour), Science Translational Medicine, June 2020. PMID: 32554708.
   Describes the development of a genetic adjuvant to enhance the generation of antiviral T cells encoded in viral vectors in mice and human studies.

3. Swadling L, Capone S, Antrobus RD, Brown A, Richardson R, Newell EW, Halliday J, Kelly C, Bowen D, Fergusson J, Kurioka A, Ammendola V, Del Sorbo M, Grazioli F, Esposito ML, Siani L, Traboni C, Hill A, Colloca S, Davis M, Nicosia A, Cortese R, Folgori A, Klenerman P, **Barnes E**. A human vaccine strategy based on chimpanzee adenoviral and MVA vectors that primes, boosts, and sustains functional HCV-specific T cell memory. ***Sci Transl Med***. 2014 Nov 5;6(261)
   Detailed T cell function and phenotype in humans after heterologous Ad/MVA prime boost vaccination

4. Kronsteiner B, Chaichana P, Sumonwiriya M, Jenjaroen K, Chowdhury FR, Chumseng S, Teparrukkul P, Limmathurotsakul D, Day NPJ, Klenerman P, **Dunachie SJ**. Diabetes alters immune response patterns to acute melioidosis in humans. Eur. J. Immunology 2019, April 29.

Characterisation of the impact of diabetes status on immune responses to Gram negative sepsis, of high relevance in understanding immune susceptibility to severe COVID-19 disease.

5. Jenjaroen K, Chumseng S, Sumonwiriya M, Ariyaprasert P, Chantratita N, Sunyakumthorn P, Hongsuwan M, Wuthiekanun V, Fletcher HA, Teparrukkul P, Limmathurotsakul D, Day NP, **Dunachie SJ**. T-Cell Responses Are Associated with Survival in Acute Melioidosis Patients. PLoS Neg Trop Dis. 9(10):e0004152. (2015).
   This work was the first to demonstrate the key role played by T cells in host defence against Gram negative sepsis from *Burkholderia pseudomallei.*

Project 1-2

Project Title: Defining the hepatic micro-environment in HBV infection under drug treatment

Supervisor: Professor Paul Klenerman - paul.klenerman@medawar.ox.ac.uk

Project Overview

Hepatitis B Virus infection is a huge public health problem with around 300 million globally infected, and high rates of carriage in China. The ability to functionally *cure* HBV once chronic infection is established is quite limited with current agents (antiviral drugs and interferon). There is much interest in developing novel approaches to cure HBV rather than just suppress it in those who require intervention. However, these approaches are hampered by a lack of understanding of the status of virus infection in the liver – i.e. what are the host responses within hepatocytes and from CD8+ T cells. This in turn has been limited by access to liver tissue. We have recently established the use of fine needle aspiration approaches to safely access liver tissue in patients with liver disease – this provides both CD45+ immune cells and CD45- parenchymal cells for study. Single cell approaches are now available to probe the transcriptional activity of cell populations in the several thousand and informatics techniques provide the possibility to define key features of critical cell populations.

In this study we will address

1. The host response in hepatocytes according to infection status of the cell and the patient.
2. The broad host responses in the host and the status of the CD8+ T cell compartment in relation to infection status
3. The relationship between the host immune response and the hepatocyte response in patients on suppressive therapy vs untreated disease states.

This information may not only help guide new strategies for cure but also guide therapy in those with chronic infection.

Training opportunities

Cellular immunology; FACS; Cell sorting; Transcriptomics; Virology; Informatics

Supervisors

Paul Klenerman - Professor of Gastroenterology/Immunology – interest in persistent virus infections and host T cell responses
Ellie Barnes Professor of Hepatology – interest in viral hepatitis and vaccines
Phillippa Matthews Associate Professor/Honorary consultant in infectious diseases – interests in HBV and molecular virology.

Key publications

1. MATTHEWS PC, CARLSON JM, BELOUKAS A, MALIK A, JOOSTE P, OGWU A, SHAPIRO R, RIDDELL L, CHEN F, LUZZI G *et al*. 2016. HLA-A is a Predictor of Hepatitis B e Antigen Status in HIV-Positive African Adults. *J Infect Dis*, **213** (8), pp. 1248-1252.

2. ANSARI MA, PEDERGNANA V, L C IP C, MAGRI A, VON DELFT A, BONSALL D, CHATURVEDI N, BARTHA I, SMITH D, NICHOLSON G *et al*. 2017. Genome-to-genome analysis highlights the effect of the human innate and adaptive immune systems on the hepatitis C virus. *Nat Genet*, **49** (5), pp. 666-673.

3. SWADLING L, CAPONE S, ANTROBUS RD, BROWN A, RICHARDSON R, NEWELL EW, HALLIDAY J, KELLY C, BOWEN D, FERGUSSON J *et al*. 2014. A human vaccine strategy based on chimpanzee adenoviral and MVA vectors that primes, boosts, and sustains functional HCV-specific T cell memory. *Sci Transl Med*, **6** (261), pp. 261ra153.

4. BARNES E, FOLGORI A, CAPONE S, SWADLING L, ASTON S, KURIOKA A, MEYER J, HUDDART R, SMITH K, TOWNSEND R *et al*. 2012. Novel adenovirus-based vaccines induce broad and sustained T cell responses to HCV in man. *Sci Transl Med*, **4** (115), pp. 115ra1.

5. FERGUSSON JR, SMITH KE, FLEMING VM, RAJORIYA N, NEWELL EW, SIMMONS R, MARCHI E, BJÖRKANDER S, KANG Y-H, SWADLING L *et al*. 2014. CD161 defines a transcriptional and functional phenotype across distinct human T cell lineages. *Cell Rep*, **9** (3), pp. 1075-1088.

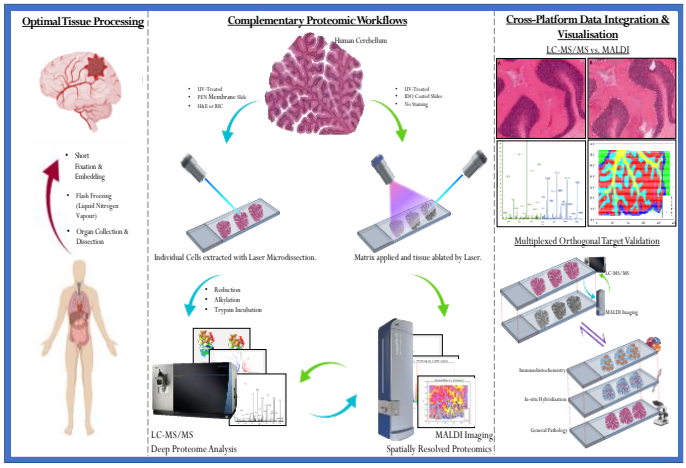
Project 2

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

Supervisor: Dr Roman Fischer - roman.fischer@ndm.ox.ac.uk

Project Overview

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



Integrated Proteomic Imaging Workflow. Pilot data leading to this application using human brain (cerebellum). Pictures are actual data. See also our paper Davis et al. (3). **Left:** We have developed a novel liquid nitrogen vapour (LNV) freezing method for large specimens and a complementary short-fixation protocol. Both are ideal for MSI. **Centre:** Left workflow – laser capture dissection of cerebellar Purkinje cells for "deep" LC-MS/MS. M/z peaks displayed. Right workflow: MALDI MSI of a serial section of the same tissue. MALDI image displayed. **Right:** Top – integration of LC-MS/MS and MALDI MSI datasets. Bottom – multiplex immunohistochemical validation of novel targets at (sub-)cellular resolution (using existing Perkin Elmer / Codex platforms at the University of Oxford).

Training opportunities

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

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

Supervisors

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

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

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

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

Professor Olaf Ansorge - I am a practising consultant neuropathologist, the director of the Oxford Brain Bank, funded by the MRC, and lead for neuropathology, genomics and biobanking of the forthcoming GBP5.8 million "Tessa Jowell BRAIN-MATRIX" trial, which aims,

for the first time, to establish a state-of-the-art tissue diagnostic infrastructure for improved brain tumour diagnostics in the UK. It comprises 10 UK centres, with Oxford acting as the molecular diagnostics hub. The ISCF and Genomics England are funding whole genome and epigenome sequencing for all recruited individuals (n=1500 over five years, start date 2020). To be able to perform MSI on a well-defined subset of these patient samples represents an ideal opportunity, as MSI data must be understood in the genetic, epigenetic and neuropathological context. Partnership with Philips Healthcare, provider of digital pathology solutions for Oxford (also ISCF funded), further enhances this studentship as it will provide the basis for digital integration of structural and MSI data on a pixel-to-pixel basis.

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

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

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

Key publications

1. Bi J, Chowdhry S, Wu S, Zhang W, Masui K, Mischel PS. Altered cellular metabolism in gliomas - an emerging landscape of actionable co-dependency targets. Nat Rev Cancer. 2020;20(1):57-70.

2. Yong C, Stewart GD, Frezza C. Oncometabolites in renal cancer. Nat Rev Nephrol. 2019.
3. 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.

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

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

Project 3

Project Title: The molecular basis underpinning Inflamm-aging

Supervisor: Professor Katja Simon - katja.simon@kennedy.ox.ac.uk

Project Overview

Age is the biggest single risk factor for many chronic diseases, from cardiovascular disease to neurodegeneration. One of the major contributing factors is chronic low-grade inflammation, also called inflamm-aging, the excess production of inflammatory cytokines. Inflamm-aging is also likely to contribute to fatal immune pathology that can occur in response to infections, particularly affecting older adults such as those suffering severe COVID19. Autophagy, a pathway activated when cells are stressed, clears away debris accumulated over time, recycling building blocks for re-use. It has been shown in model organisms and other tissues that autophagy maintains a healthy lifespan and a young immune system 1. Our hypothesis is that declining autophagy levels with age underpin ageing of the immune system, in particular inflamm-aging. We will focus on macrophages in this project, the main inflammatory cell in the body.

We have discovered that autophagy prevents premature aging of the macrophage 2. We have also found in lymphocytes that autophagy decreases with age. Here, we will investigate to what extent this contributes to inflamm-aging in macrophages, and then identify in which type of macrophages (tissue-resident versus bone marrow-derived) autophagy prevents inflamm-aging. Then we aim to understand what autophagy needs to degrade to prevent inflamm-aging in macrophages. For this, we have successfully generated a novel mouse model, in which the autophagic cargo is directly proximity-labelled in primary cells (unpublished). The cargo may for example include dysfunctional mitochondria or the machinery that makes inflammatory cytokines (inflammasome). This knowledge will help us to better understand the dysfunctional cell biology behind the cellular aging process.

Our second aim is to understand the contribution of the microenvironment in which macrophages live to inflamm-aging. The adipose tissue contains large numbers of macrophages. During aging, adipose tissue increases, which is known to contribute to increased inflammation, however, whether this is via autophagy has not been studied. We have found that autophagy of the adipose cells themselves prevents inflammation, by providing nutrients to the anti-inflammatory macrophages, while in the absence of autophagy in adipose tissue the inflammatory macrophages prevail. Here we are to explore if this contributes to inflamm-aging. We will measure if there is an age-related decline in autophagy in adipose tissue, what exactly does autophagy provide to the neighbouring macrophage (for example fatty acids which are stored by adipose cells) 3 and can those be used to reverse inflamm-aging in macrophages.

Lastly, we will address whether human adipose tissue (obtained through OCDEM biobank) and macrophages (ethics are in place for PBMCs from older adults) also show a decline in autophagy. By using tissue explants and co-culture systems, we will address whether macromolecules provided via autophagy can reverse any ageing phenotype.

Treating age-related illnesses puts health care systems under enormous economic pressure. Novel pathways targeting processes underlying age-related metabolic and molecular damage provide an opportunity to prevent or slow the emergence of chronic pathologies.

Training opportunities

Training will be provided in techniques including proteomics and RNAseq and their analysis, confocal microscopy, immunological techniques such as multi parameter flow cytometry. You will attend regular seminars within the department and in the wider University. You will be expected to present data regularly in lab meetings and in departmental progress report seminars and in national and international conferences. You will have the opportunity to work closely with collaborating groups interested in inflammation (Fiona Powrie, KIR, Jelena Bezbradica-Mirkovic, KIR, Claudia Waskow, Jena, Christian Behrends, Munich, Fred Karpe, OCDEM). A senior postdoc in the lab will initially supervise you. A core curriculum of lectures is offered in the first term to provide a solid foundation in a broad range of subjects including inflammation, epigenetics, translational immunology, data analysis, statistics and the microbiome.

Environment

The Kennedy Institute is a world-renowned research centre, housed in a brand new, state-of-the-art facility at the University of Oxford under the directorship of Fiona Powrie. The Simon lab consists currently of 4 postdocs, 3 DPhil students (two in their final year and one first year) and we will recruit a bioinformatician and research assistant with recently funded Wellcome award. It is a small, friendly and very international lab. While lab members are ambitious to work each on different projects, team work is very much encouraged. We strive to give every DPhil student the opportunity to write a review and publish a first author primary paper. We regularly welcome Master and other short-term students in the lab, so there will be opportunities to train your supervision skills.

Supervisor short profile

Katja Simon is Professor at Oxford University and Principal Investigator at the Kennedy Institute of Rheumatology. She trained as an Immunologist under Avrion Mitchison at the DRFZ Berlin and showed in her PhD that TH1 cytokines are found in excess in rheumatoid arthritis (EULAR Award). As a postdoc at the Centre d'Immunologie Marseille Luminy and Oxford she pursued an interest in cell fate, studying cell death molecules in thymic selection, inflammation and tumour immunity. As a Principal Investigator, she turned her attention to autophagy, and discovered that autophagy maintains healthy red blood cells, stem cells and memory T cells. It promotes differentiation and prevents ageing of the hematopoietic system. She is a Wellcome investigator (2021-2026) and recipient of the European Ita Askonas award 2018 for outstanding European female group leaders in Immunology.

Key Publications

1. Zhang H, Puleston DJ, Simon AK. Autophagy and Immune Senescence. Trends Mol Med 2016; 22:671-86.

2. Stranks AJ, Hansen AL, Panse I, Mortensen M, Ferguson DJ, Puleston DJ, et al.,.. Simon AK. Autophagy Controls Acquisition of Aging Features in Macrophages. J Innate Immun 2015; 7:375-91.

3. Riffelmacher T, Clarke A, Richter FC, Stranks A, Pandey S, Danielli S, et al. ….Simon AK. Autophagy-Dependent Generation of Free Fatty Acids Is Critical for Normal Neutrophil Differentiation. Immunity 2017; 47:466-80 e5.

4. Zhang H, Alsaleh G, Feltham J, Sun Y, Napolitano G, Riffelmacher T, et al. ….Simon AK. Polyamines Control eIF5A Hypusination, TFEB Translation, and Autophagy to Reverse B Cell Senescence. Mol Cell 2019; 76:110-25 e9.

5. Alsaleh G, Panse I, Swadling L, Zhang H, Meyer A, Lord J, et al…. Simon AK Translational control of autophagy is key to T cell vaccine responses in older adults.biorxiv eLife; under review.

Project 4

Project Title: Genomics of host susceptibility to severe infection

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

Project Overview

This proposal aims to provide training in genomic medicine with particular application to immunology and infectious disease, combining a high-quality scientific research project focused on investigating the genomics of susceptibility to severe infection with clinical experience in genomic medicine and internal medicine in the Oxford University Hospitals NHS Trust. The research will be collaborative, based in Oxford at the laboratory of Professor Julian Knight at the Wellcome Centre for Human Genetics while working closely with Professor Jianwei Wang at Peking Union Medical College to enable access to expertise and clinical samples from patients in China to complement those patients recruited from the UK that will be required for the proposed research.

Research proposal

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

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

This research project will address the question of why some patients develop severe infections focusing on sepsis, COVID-19 and related infections. This will involve using genomics to investigate the role of genetic variation in disease, combining analysis of particular rare and common DNA sequence variants with functional genomic studies to understand the consequences of such variation for immune function and clinical outcome.

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

We have discovered that distinct patterns of leukocyte gene expression occur in adult sepsis patients (sepsis response signatures, SRS). These define specific novel disease endotypes that are informative for the underlying immune response state and outcome, that are robust to source of infection and have been independently validated. We further find that membership of disease endotypes cannot be established from clinical covariates and in a subset of patients are dynamic over time. Importantly, these sepsis endotypes predict response to therapy.

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

We have an ongoing major programme of work in COVID-19 in Oxford involving deep phenotyping and in collaboration with Professor Wang.

Research plan

1. To identify genetic factors associated with disease endotypes. Here, we will analyse patients from the UK GAinS cohort and assign disease endotypes for individual patients based on quantifying a diagnostic gene expression signature. We will use genome-wide sets of genetic markers (single nucleotide polymorphisms) to establish association with disease endotypes and then proceed to investigate the relationship with disease outcome and susceptibility. We hypothesise that, in contrast to GWAS for all sepsis patients, reducing disease heterogeneity by analysing specific disease endotypes will result in significantly increased numbers of associations. We will then functionally characterise these genetic associations using established methods in the Knight lab to characterise regulatory genetic variants. Here, association with differences in gene expression can be identified based on stored RNA and plasma together with functional genomic methods such as genome engineering (CRISPR/Cas9) and analysis of chromatin regulation and conformation. Over the course of the D.Phil, Professors Knight and Wang will be establishing a new prospective cohort of patients with sepsis from China on whom a similar analysis will be performed. We will also explore how genetic factors may predispose to COVID-19 disease endotypes.

2. To define the role of rare highly penetrant mutations in susceptibility to severe infection. Here we will use whole exome and whole genome sequencing, applied to young patients with severe infections such as sepsis and COVID-19 who have no predisposing factors, together with other patients with unusual severe infections such as invasive pneumococcal disease or where primary immunodeficiency is suspected but no cause identified through conventional clinical testing. We will analyse probands and parents in a trio design where feasible, using established analytical pathways in the Knight lab. We will work with Professor Wang to recruit patients with these phenotypes in China, performing whole exome and whole genome sequencing. Functional follow up will be enabled by collaboration with colleagues in immunology and structural biology in Oxford.

Training Opportunities
The clinician undertaking this project will gain a comprehensive research training in genomics together with related expertise in immunology and infectious disease. This will include training in bioinformatics and statistical genetics as well as functional genomics. The student will benefit from relevant modular teaching through the Genomic Medicine and Statistics DPhil programme (for which Professor Knight is the Course Director) and the Medical Sciences Doctoral Training Centre. Clinical training in internal medicine will be enabled through attachment to a clinical firm at the John Radcliffe Hospital under the supervision of Professor Knight (Honorary Consultant) for one month per year. The student will also benefit from attendance at Genomic Medicine Multi-Disciplinary Team meetings (chaired by Professor Knight) which are focused on rare disease and are cross-disciplinary; the trainee will also benefit from interaction with clinical scientists involved in analysis rare variants for example through the UK 100,000 Genomes Project.

Supervisors:
Primary: Professor Julian Knight
CAMS co-supervisor: Professor Jianwei Wang

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

Key publications

1. Ellinghaus D, et al. 2020 Genomewide Association Study of Severe Covid-19 with Respiratory Failure. *New England Journal of Med*icine. doi: 10.1056/NEJMoa2020283

2. Zhang Y, Qin L, Zhao Y, Zhang P, Xu B, Li K, Liang L, Zhang C, Dai Y, Feng Y, Sun J, Hu Z, Xiang H, Knight JC, Dong T, Jin R. 2020 Interferon-Induced Transmembrane Protein 3 Genetic Variant rs12252-C Associated With Disease Severity in Coronavirus Disease 2019. *Journal of Infectious Diseases* **222**, 34-37.

3. Antcliffe DB, Burnham KL, Al-Beidh F, Santhakumaran S, Brett SJ, Hinds CJ, Ashby D, Knight JC, Gordon AC. 2018 Transcriptomic Signatures in Sepsis and a Differential Response to Steroids: From the VANISH Randomized Trial. *American Journal of Respiratory and Critical Care Medicine* **199**, 980-986

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

5. Davenport EE, Burnham KL, Radhakrishnan J, Humburg P, Hutton P, Mills TC, Rautanen A, Gordon AC, Garrard C, Hill AV, Hinds CJ & Knight JC. 2016 Genomic landscape of the

individual host response and outcomes in sepsis: a prospective cohort study. *Lancet Respir Med* **4**: 259-71.

6. Fairfax BP, Humburg P, Makino S, Naranbhai V, Wong D, Lau E, Jostins L, Plant K, Andrews R, McGee C & Knight JC. 2014 Innate immune activity conditions the effect of regulatory variants upon monocyte gene expression. *Science* **343**: 1246949.

7. Parks T, Elliott K, Lamagni T, Auckland K, Mentzer AJ, Guy R, Cartledge D, Strakova L, Connor DO, Pollard AJ, Neville MJ, Mahajan A, Ashrafian H, Chapman SJ, Hill AVS, Sriskandan S & Knight JC. Elevated risk of invasive group A streptococcal disease and host genetic variation in the human leucocyte antigen locus. *Genes Immun* 2019 **21**, 63-70.

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

Projects 5

Project Titles

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

Supervisor: Professor Doug Higgs - 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

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

1. Single-Cell Proteomics Reveal that Quantitative Changes in Co-expressed Lineage-Specific Transcription Factors Determine Cell Fate. Palii CG, Cheng Q, Gillespie MA, Shannon P, Mazurczyk M, Napolitani G, Price ND, Ranish JA, Morrissey E, **Higgs DR**, Brand M. Cell Stem Cell. 2019

2. Tissue-specific CTCF-cohesin-mediated chromatin architecture delimits enhancer interactions and function in vivo. Hanssen LLP, Kassouf MT, Oudelaar AM, Biggs D, Preece C, Downes DJ, Gosden M, Sharpe JA, Sloane-Stanley JA, Hughes JR ...**Higgs DR** 2017. *Nat Cell Biol* **19**: 952-961.
3. Testing the super-enhancer concept by in-vivo dissection. Hay D, Hughes JR, Babbs C, Davies JOJ, Graham BJ, Hanssen L, Kassouf MT, Oudelaar AM, Sharpe ja, Suciu M, Telenius J, Williams R, Rode C, Li P-S, Pennacchio LA, Sauka-Spengler T, Sloane-Stanley JA, Ayyub H, Butler S, Gibbons RJ, Smith AJH, Wood WG & **Higgs DR** (2016) *Nature genetics*, **48**, 895-903.
4. Analysis of hundreds of cis-regulatory landscapes at high resolution in a single, high-throughput experiment. Hughes, J.R., Roberts, N., McGowan, S., Haay, D., Giannoulatou, E., Lynch, M., de Gobbi, M., Taylor, S., Gibbons, R. & **Higgs, D.R**. (2014) *Nat Genet*, **46**: 205-212.

5. Dynamics of the 4D genome during in vivo lineage specification and differentiation. Oudelaar AM, Beagrie RA, Gosden M, de Ornellas S, Georgiades E, Kerry J, Hidalgo D, Carrelha J, Shivalingam A, El-Sagheer AH, Telenius JM, Brown T, Buckle VJ, Socolovsky M, **Higgs DR**, Hughes JR. Nat Commun. 2020

Project 6

Project Title: Immunopathogenesis of Covid19 in the lung

Supervisor: Professor William James - william.james@path.ox.ac.uk

Project Overview

In the great majority of cases, infection of the ACE2-rich epithelia of the upper airways by SARS-CoV-2 results in a self-limiting and mild disease that transmits epidemically by aerosol and droplets. In a small proportion of cases, and particularly in the elderly and those with metabolic and inflammatory diseases, the virus descends to the alveoli, where infection of type 2 pneumocytes and alveolar macrophages is followed by interstitial pneumonitis and mononuclear cell infiltration. Even in patients that recover from Covid19 pneumonia, *sequelae* involving microthrombi and inflammation at multiple extrapulmonary sites – probably in the absence of local virus replication – often result in long-term disability.

As part of the global effort to understand this pathological switch, we have established an experimentally tractable and physiologically authentic "alveolus-in-a-dish" model using human pluripotent stem-cell-derived Type2 pneumocytes and macrophages. The student will join a team of stem cell technologists and virologists with a track record in both hiPSC tissue models and SARS-CoV-2 to test key hypotheses concerning the molecular and cellular mechanism of the immunopathological "switch". They will benefit from established collaborations between the host laboratory and the networks of immunologists and physiologists that have been established in relation both to Covid19 and neuroinflammatory disease.

By October 2021, many critical questions in the field will have been resolved, and others will have arisen, meaning that the project will need to remain flexible until shortly before it commences. Nevertheless, currently open questions provide a useful indication of the likely project:

1. To what extent do antibodies possess the potential to case an enhancement (ADE) of viral infection and/or exacerbation of inflammatory responses to infection in the alveolus-in-a-dish? Antibodies would include non-neutralizing mAbs, neutralizing mAbs at sub-neutralizing concentration, polyclonal convalescent sera, and sera from vaccine trial participants. The effect of isotype, FcR-binding, complement activation, etc will be examined.
2. To what extent do non-infectious RNP complexes derived from vRNA fragments and host proteins ("*toxic flotsam*™") found in patient tissues provoke inflammatory responses in the model?
3. To what extent can primary Th cells from convalescent subjects respond to virus-infected, of virus- antigen-pulsed can MHC Class II-matched alveolar macrophages? What potentially pathological reactions do such responses provoke in pneumocytes?
4. To what extent do widely available drugs, such as Beclometasone, Budesonide, and so on, ameliorate the potentially pathological responses identified above?

Training opportunities
The student will:

1. receive a comprehensive training in human pluripotent stem cell technology, including propagation, quality control, differentiation (macrophage, neuron, pneumocyte) and use (2Dand 3D models) in the James & Lillian Martin Centre for Stem Cell Research, directed by the supervisor;
2. be trained in safe and effective working methods for both hazard group 2 viruses (such as lentivirus vectors and ZIKV) and hazard group 3 SARS-CoV-2 in the core virus facility directed by the supervisor. They would learn how to propagate, titrate and sequence virus, and how to evaluate the potency of antiviral agents;
3. be trained in high content confocal image analysis (OperaPhenix), flow cytometry (including ImageStream), ELISA, ELIspot and other relevant immunoassays
4. learn standard, research-level methodologies in molecular and cell biology
5. receive advanced training in data analysis, including bioinformatics and digital image analysis
6. be trained in strict adherence to data integrity practice.

Supervisor
William James 詹衛亮 (https://orcid.org/0000-0002-2506-1198) is an academic with an international profile for innovative medical research and teaching and has held leadership roles within the University of Oxford including most recently senior Pro-Vice Chancellor (Planning and Resources).

He has run an independent research laboratory at the Sir William Dunn School of Pathology since 1984, investigating the biology of HIV-1 and developing novel methods for its control. In summary he has made significant contributions to virology, particularly in relation to the pathway of virus entry into host cells, and especially the interaction between HIV-1 and macrophages. In the process, he has developed cutting-edge technologies that are in wide use by medical scientists worldwide, including antiviral aptamers and stem-cell-based tissue models for infection and inflammation. Most recently, he has been able to provide core virology expertise for the Covid19 community, contributing SARS-CoV-2 isolation and characterization from clinical samples, and determining the neutralizing potency of human sera (including vaccine trial participants), monoclonals, nanobodies, chimeric proteins and aptamers.

He has received awards for University teaching and published in the field of medical education. He was co-developer of the Biomedical Admissions Test (BMAT; run by Cambridge Assessment), authored the award-winning, JISC-funded iCase system for rich online learning environments, pioneered the use of PBL within Oxford tutorials, and published a Small Private Online Course (SPOC) on evolution for medical students. He is a Trustee of the educational charity, the Oxford Trust, and is Editor-at-Large for the media channel Voices from Oxford.
He has mentored dozens of undergraduate and graduate students, post-docs and academics, many of whom have reached eminent positions in the academy. He currently heads a 14-strong laboratory, supported by programme-level funding, and was submitted for the 2013 REF, along with two junior academic colleagues in his group.

Clinical supervisors
Chris Conlon

Key publications

1. Carter G, Bernstone L, Sangani D, Bee J, Harder T, ***James W*** (2009) HIV entry in macrophages is dependent on intact lipid rafts. Virology 386:192–202 . https://doi.org/citeulike-article-id:4168265 doi: 10.1016/j.virol.2008.12.031

2. Haenseler W, Sansom S, Buchrieser J, Newey S, Moore C, Nicholls F, Chintawar S, Schnell C, Antel J, Allen N, Cader Z, Wade-Martins R, ***James W***, Cowley S (2017) A Highly Efficient Human Pluripotent Stem Cell Microglia Model Displays a Neuronal-Co-culture-Specific Expression Profile and Inflammatory Response. Stem Cell Reports 8:1727–1742 . https://doi.org/citeulike-article-id:14374435 doi: 10.1016/j.stemcr.2017.05.017

3. Huo J, Le Bas A, Ruza RR, Duyvesteyn HME, Mikolajek H, Malinauskas T, Tan TK, Rijal P, Dumoux M, Ward PN, Ren J, Zhou D, Harrison PJ, Weckener M, Clare DK, Vogirala VK, Radecke J, Moynié L, Zhao Y, Gilbert-Jaramillo J, Knight ML, Tree JA, Buttigieg KR, Coombes N, Elmore MJ, Carroll MW, Carrique L, Shah PNM, ***James W***, Townsend AR, Stuart DI, Owens RJ, Naismith JH (2020) Neutralizing nanobodies bind SARS-CoV-2 spike RBD and block interaction with ACE2. Nat Struct Mol Biol 1–9 . https://doi.org/10.1038/s41594-020-0469-6

4. Karlsson KR, Cowley S, Martinez FO, Shaw M, Minger SL, ***James W*** (2008) Homogeneous monocytes and macrophages from human embryonic stem cells following coculture-free differentiation in M-CSF and IL-3. Exp Hematol 36:1167–1175 . https://doi.org/10.1016/j.exphem.2008.04.009

5. Khati M, Schüman M, Ibrahim J, Sattentau Q, Gordon S, ***James W*** (2003) Neutralization of Infectivity of Diverse R5 Clinical Isolates of Human Immunodeficiency Virus Type 1 by gp120-Binding 2'F-RNA Aptamers. J Virol 77: . https://doi.org/10.1128/JVI.77.23.12692-12698.2003

Project 7

Project Title: Immunosenescence; Exploration of Vaccine induced Immune Response in Older Adults

Supervisor: Professor Teresa Lambe - teresa.lambe@ndm.ox.ac.uk

Project Overview

The Oxford Vaccine Centre's COVID-19 vaccine trial is being run by the Jenner Institute and Oxford Vaccine Group with the results of the Phase I/II trial being recently published in The Lancet which indicate an acceptable safety profile and the induction of a strong cellular and antibody responses. The vaccine provoked a T cell response within 14 days of vaccination and an antibody response within 28 days with a second dose increasing the antibody titres.
Diminished antibody titers have been measured in older adults post vaccination and, in many cases, the quality of these antibody responses is inferior compared to younger adults. The cellular compartment is also affected during aging, with contraction of the naïve T cell repertoire and accumulation of terminally differentiated cell subsets frequently with altered effector functions.

A key target group for a SARS CoV-2 vaccine are older adults. We have tested the immunogenicity of the adenoviral vectored vaccine ChAdOx1 nCoV-19 (AZD-1222) in aged mice and find that a single dose of this vaccine induces cellular and humoral immunity but at a reduced magnitude when compared to younger mice. A second dose enhances the immune response, indicating that a prime-boost strategy may be desirable to enhance immunogenicity in older adults.

Within the clinical programme we have started to vaccinate older adults and assess the immunogenicity of ChAdOx1 nCoV-19 (AZD-1222). We propose to build on these works through this doctoral training programme and examine in detail the immune response in older adults, including antibody functionality, cellular immune responses (e.g. follicular T cells responsiveness) and Cytomegalovirus (CMV) status.

Training opportunities

Both specialised subject training and generic research capabilities will be developed.
Including but not limited to:

- Immunogenicity assessment of human vaccine samples
- Cellular immune assays (ELISpot, AIM, FACS & Intracellular Cytokine Staining (ICS))
- Humoral immune assays (ELISA, FACS & Cultured ELISpot)
- Development of translational assays (e.g. Pseudotyped virus assay, systems serology)
-

All students will be expected to analyse, interpret and present their data internally and at appropriate conferences. This project will provide a broad range of transferable skills with a unique insight into translational research.

Supervisor
Associate Professor Lambe is based at the Jenner Institute, University of Oxford, wherein her research programme focuses on developing innovative vaccines against emerging and outbreak pathogens.

Professor Lambe played a key role in the initial design of the ChAdOx1 nCoV-19 vaccine and is now overseeing the immunology of the ChAdOx1 nCoV-19 clinical programme. Outside of her most recent work on the SARS-CoV-2 vaccine, her group have been progressing the clinical development of a novel vaccine against Ebolavirus, as well as profiling how the immune system responds post-exposure to viruses causing lethal haemorrhagic disease.

Clinical supervisors
Professor Andrew Pollard
Other PI's involved Professor Sarah Gilbert

Key publications

1. Ewer, K., et al. Chimpanzee adenoviral vectors as vaccines for outbreak pathogens. Hum Vaccin Immunother 13, 3020-3032 (2017).

2. Folegatti, P.M., et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. Lancet 396, 467-478 (2020).

3. Graham, S.P., et al. Evaluation of the immunogenicity of prime-boost vaccination with the replication-deficient viral vectored COVID-19 vaccine candidate ChAdOx1 nCoV-19. NPJ Vaccines 5, 69 (2020).

4. van Doremalen, N., et al. A single dose of ChAdOx1 MERS provides protective immunity in rhesus macaques. Sci Adv 6, eaba8399 (2020).

5. van Doremalen, N., et al. ChAdOx1 nCoV-19 vaccine prevents SARS-CoV-2 pneumonia in rhesus macaques. Nature (2020).

Project 8-1

Project Title: Discovery of potent wild-type and surrogate agonist peptides for anti-tumor T-cell receptors

Supervisor: Dr Ricardo Fernades - ricardo.fernandes@ndm.ox.ac.uk

Project Overview

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 tumor cells is determined by the signaling 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 strong agonist peptides is still notoriously challenging. The aim of this project is to establish a framework to identify potent agonist peptides recognized by effector and regulatory T cells of interest, with a strong focus in identifying peptides recognized by TCRs from expanded tumor infiltrating lymphocytes.

The identification of antigens recognized by the TCR has been challenging given the extreme diversity of the three individual components involved: peptide antigens, TCR and MHC. Our aim is to identify peptides, neoantigens and mimotopes, recognized by the TCR of clonally expanded CD8+ effector and CD4+ T cells in tumor settings. 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 recognized by TCRs of interest. This affinity-based approach will be complemented by a functional screen using an engineered system in mammalian cells, whereby the peptide-MHC library is fused to a CAR-like signaling module displayed by T cells. This functional-based selection hijacks the unique sensitivity and specificity of the CD28/CD3 signaling 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 and the discovery of self-peptides or neoantigens using custom built algorithms to identify closely related wild-type peptides. The identification of peptides recognized by T cells of interest will further enable the production of tumor-specific peptide-MHC tetramers to be used in T cell isolation for detailed phenotypic characterization using a wide-range of techniques such as single-cell transcriptomics and proteomics. 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-tumor immune response. The outcome of this research is expected to contribute towards a better understanding of T cell function and to the development of relevant immunotherapies in cancer settings. Furthermore, the discovery of disease-related agonist peptides opens the possibility to modulate T cell activity by peptide immunization, an important first step towards achieving in vivo expansion and activation of tumor-specific T cells.

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

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

Clinical Supervisor
Professor Chris Conlon, Professor of Infectious Diseases, Head of NDM Experimental Medicine

Key Publications

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

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

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

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

Project 8-2

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

Supervisor: Dr Ricardo Fernades - 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 therapy, suggesting that a fundamental understanding of the basic mechanisms of immune checkpoint receptor signaling 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.

Lymphocytes have developed a highly sophisticated signaling apparatus to detect extracellular cues using surface receptors that signal via immunoreceptor tyrosine-based motifs (e.g. ITAM, ITIM and ITSMs). These signaling motifs relay information from multiple, structurally diverse, receptors such as the T-cell receptor (TCR), Fc receptor (FcR, B-cell receptor, CD28, PD-1 and Signal-regulatory protein SIRP, among others. The ITAM/ITIM-signaling mechanism is unique, in that it relies on tyrosine phosphorylation induced by members of the Src kinase family, such as Lck or Fyn and phosphorylation is regulated by multiple processes, including by the opposing action of phosphatases, such as CD45. While ligand binding promotes receptor phosphorylation, growing evidence suggests that tonic, ligand-independent, signaling is an important property of this class of signaling motifs and has been observed for the TCR, Fc receptor, SIRP, CTLA-4, CD28, and PD-1.

Regulation of T cell signaling by immune checkpoints such as PD-1 and CTLA-4, has been at the center of recent breakthroughs in cancer immunotherapy. Signaling 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 signaling motifs and results in the recruitment of cytosolic phosphatases such as SHP1/2, which in turn reduce TCR and CD28 signaling. Strikingly, signaling by several immune receptors relies on the Tyr phosphorylation of ITAM/ITIM/ITSM signaling motifs. We hypothesize that tonic receptor phosphorylation and sustained signaling 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, to within close

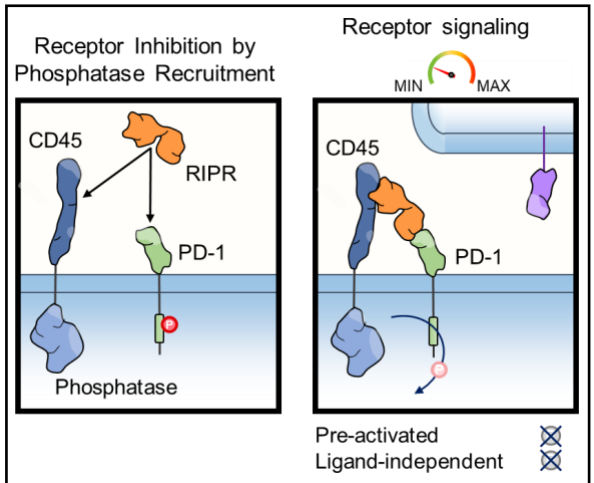


Fig. 1 | Receptor Inhibition by Phosphatase Recruitment. RIPR combines checkpoint blockade with CD45 recruitment. CD45 dephosphorylates receptors such as PD-1.

proximity of PD-1. In this approach, the intracellular phosphatase domain of CD45 acts intracellularly, in *cis,* on the p-Tyr residues of the PD-1 ITIM/ITSM motif, thus inhibiting sustained signaling. 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 tumor growth in mouse models of small cell lung cancer (SCLC) and colon adenocarcinoma (MC38) (Fig. 2; Fernandes *et al.*, Nature, *in press*). Here, we propose the development of the RIPR concept to dissect the role of PD-1 in T cell "exhaustion" and, in parallel, to expand this novel approach to target other key immune and cancer-specific receptors aimed at generating novel antitumor, RIPR-based, molecules.

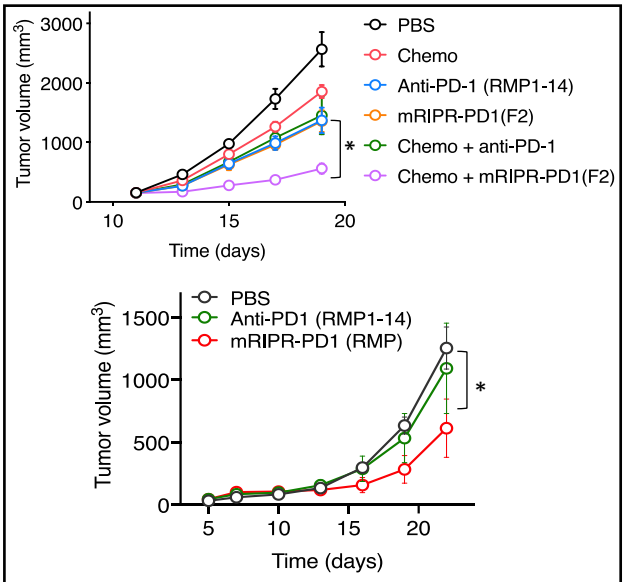


Fig. 2 | Combination of RIPR-PD1 with cisplatin and etoposide (chemo) is superior to chemo and anti-PD1 antibody in a SCLC model (*top; N*=5, representative of 2 repeats). RIPR-PD1 is superior to anti-PD1 in a colon adenocarcinoma tumor model (*bottom; MC38; N*=10, representative of 2 repeats).

Aim 1. Determining a high-resolution, longitudinal, transcriptome of tumor infiltrating lymphocytes (TILs) upon RIPR-PD-1 treatment. Despite recent advances, the mechanistic basis of PD-1 inhibition remains incomplete. Reversal of the T cell exhaustion phenotype by antibody blockade appears to be inefficient. *Is T cell exhaustion irreversible?* RIPR-PD1 offers a new avenue to investigate the role of PD-1 and offers several advantages over genetic deletion and antibody blockade, such as: p-Tyr-specificity, controlled delivery and tunability. For this aim we propose to sequence TILs, at different stages of an antitumor response and contrast this to the transcription profile upon RIPR-PD1 treatment. A longitudinal map will be created focusing on information obtained from single-cell transcriptome, chromatic accessibility (ATAC-seq) and TCR sequencing. The unbiased understanding of T cell dynamics before and after exposure to RIPR-PD1 will elucidate which pathways are turned "on" or "off" by PD-1 signaling. This information will help determining a roadmap to investigate the mechanistic basis of immune checkpoint function with unparalleled granularity, and in the future will enable the development of novel therapeutic modalities.

Aim 2. Development of RIPR-based molecules to inhibit immune receptors. Currently, we have developed RIPR-based molecules for PD-1 and SIRP□□□Fernandes *et al.*, Nature,□*in press*□. However, other immune checkpoint receptors play important inhibitory roles. *Can the RIPR concept be applied to other inhibitory receptors?* We propose the development of a platform to systematically probe the RIPR effect in key immune inhibitory receptors. We will develop RIPR molecules to target other molecules of interest. For this, we will use publicly available antibody sequences or isolate scFv or nanobodies from yeast libraries. Following *in vitro* testing of RIPR activity, promising candidates will be tested for antitumor activity *in vivo* in appropriate mouse models. Determinants of RIPR activity, such as epitope binding, receptor geometry and binding affinity, will be identified to better define the optimal RIPR architecture.

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

Training opportunities

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

Supervisor

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

Clinical supervisor

Professor Chris Conlon, Professor of Infectious Diseases, Head of NDM Experimental Medicine

Key publications

1. **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

2. 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

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

4. Garcia KC, **Fernandes RA**. May, 2018. WO/2019/222547. Receptor inhibition by phosphatase recruitment.

5. **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. (2020) Immune receptor inhibition through enforced phosphatase recruitment. Nature*, in press denotes equal contribution*

Project 9

Project Title: Discovery of host cell targets cleaved by SARS-COV2 M^{pro} and PL^{pro} proteases

Supervisor: Professor Benedikt Kessler - benedikt.kessler@ndm.ox.ac.uk

Project Overview

Background. SARS viruses encode for two proteases, the main chymotrypsin-like protease M^{pro} (3CLpro), encoded by the non-structural protein 5 (NSP5), and the papain-like protease PL^{pro} encoded by NSP3. PL^{pro} is a cysteine protease with sequence, has structural similarities to members of the papain family and can function as a deubiquitylase (DUB). In the context of host-pathogen interactions, PL^{pro} derived from SARS CoV, but also MERS CoV, also recognises and cleaves a di-ubiquitin analogue, the interferon stimulated gene 15 (ISG15), which is an integral part of the human antiviral response (Shin D et al., 2020). Also, CoV encoded papain-like proteases interfere with the host cell antiviral type I interferon pathway. M^{pro} is involved in processing the conserved polyprotein nsp7-10 region, which is essential for viral maturation.

PL^{pro}/M^{pro} cell degradomics – substrate discovery. Cellular protein targets proteolytically processed by M^{pro} and PL^{pro} have not yet been explored at a systematic level. Analytical workflows based on 'degradomics' have been developed, such as N-terminomics of amino-termini using isotope labelling (TAILS) (Kleifeld et al., 2011) and high efficiency undecanal terminomics (HUNTER) (Weng et al., 2019). The Kessler lab has established both experimental techniques, and plans to explore M^{pro} and PL^{pro} cellular degradomics in A549 cells expressing SARS viral proteases. We shall also compare the degradomics of SARS-COV-1 versus SARS-COV-2 encoded proteases M^{pro} and PL^{pro} to discriminate factors that might contribute to the different infection dynamics of the two viruses. Individual host cell substrate proteins identified will be studied in further detail and the relevance of their proteolytic processing assessed in the context of cellular physiology.

Training opportunities

- Introduction to background biology of the cellular ubiquitin system and its function in normal physiology as well as cancer and neurodegeneration

- Cell culture, transfection techniques, immunoprecipitation, SDS-PAGE and western blotting

- Sample preparation techniques for mass spectrometry analysis including tandem mass tagging (TMT), in-solution and in-gel digestion, HPLC pre-fractionation and sample desalting

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

- Introduction to bioinformatics tools to process –omics data, such as R (training courses) and more specialised –omics analysis software including Mascot, MaxQuant, Perseus, SAINT, Progenesis IQ, Proteomics Discoverer, PEAKS, MS Fragger, Fragpipe)

Supervisor
Benedikt Kessler group is focused on ubiquitin and protease biology with a specialty in mass spectrometry, proteomics and recently in metabolomics. Expertise in his laboratory is also used to define “molecular signatures” in disease processes and accelerate target discovery in translational research. The Kessler Lab is currently developing chemoproteomics methods to profile active ubiquitin processing enzymes, in particular deubiquitylating enzymes (DUBs) and the dynamic ubiquitome. Ubiquitin-based active site directed probes were developed that allowed the profiling of the active cellular content of the DUB enzyme family. This approach was also used to demonstrate the involvement of otubain 1 (OTUB1) in infection and prostate cancer, the role of USP4 and USP4 in DNA repair mechanisms and the characterisation of USP7 inhibitors as novel potential therapeutic agents in multiple myeloma.

Clinical supervisors
Prof Alison Simmons (WIMM, Oxford, UK)
Prof Julian Knight (WCHG, Oxford, UK)

CAMS PI
Prof Chengyu Jiang (PUMC)

Key publications

1. Shin D, Mukherjee R, Grewe D, Bojkova D, Baek K, Bhattacharya A, Schulz L, Widera M, Mehdipour AR, Tascher G, Geurink PP, Wilhelm A, van der Heden van Noort GJ, Ovaa H, Müller S, Knobeloch KP, Rajalingam K, Schulman BA, Cinatl J, Hummer G, Ciesek S, Dikic I. Papain-like protease regulates SARS-CoV-2 viral spread and innate immunity. Nature. 2020 Jul 29. doi: 10.1038/s41586-020-2601-5.

2. Peng Y, Mentzer AJ, Liu G, Yao X, Yin Z, Dong D, Dejnirattisai W, Rostron T et al. Broad and strong memory CD4 + and CD8 + T cells induced by SARS-CoV-2 in UK convalescent individuals following COVID-19. Nat Immunol. 2020 Sep 4. doi: 10.1038/s41590-020-0782-6.

3. Weng, S. S. H. et al. (2019) ‘Sensitive determination of proteolytic proteoforms in limited microscale proteome samples’, Molecular and Cellular Proteomics. doi: 10.1074/mcp.TIR119.001560.

4. Emma J Fenech, Federica Lari, Philip D Charles, Roman Fischer, Marie Laétitia-Thézénas, Katrin Bagola, Adrienne W Paton, James C Paton, Mads Gyrd-Hansen, Benedikt M Kessler, John C Christianson. Interaction mapping of endoplasmic reticulum ubiquitin ligases identifies modulators of innate immune signalling. eLife. 2020; 9: e57306.

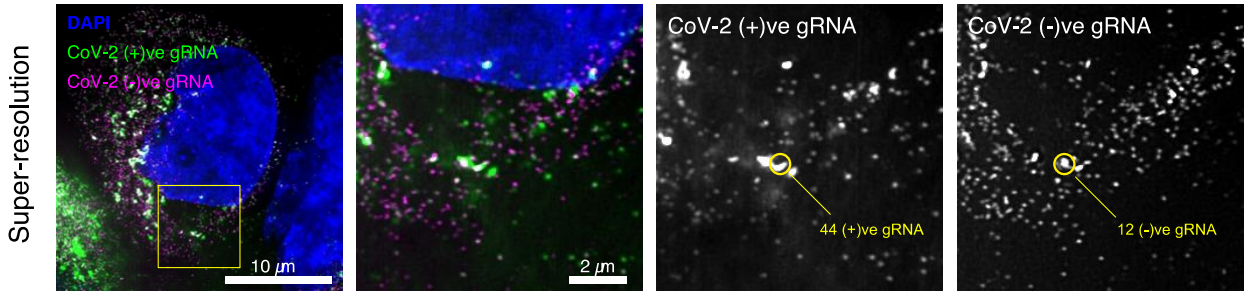
Project 10

Project Title: Super resolution imaging of SARS-CoV-2 replication and transmission

Supervisor: Professor Jane McKeating - jane.mckeating@ndm.ox.ac.uk

Project Overview

The COVID-19 pandemic, caused by the SARS-CoV-2 coronavirus, is a global health issue with more than >730,000 fatalities to date. Understanding host pathways that regulate host susceptibility to SARS-CoV-2 infection and transmission will inform clinical management of this disease. SARS-CoV-2 replicates in cells of the respiratory tract and we have developed super resolution *in situ* hybridization methods to image single molecules of SARS-CoV-2 RNA in infected cells. Detecting >95% of individual positive and negative strand viral genomes, enables us to quantify the replicative burst within the first hours of infection and to track viral replication and transmission. This transformational methodology will provide an unprecedented sensitivity and spatial precision to define viral tropism and to identify the viral reservoir within clinical material.



Single-molecule imaging of SARS-CoV-2 viral RNAs. *Calu-3 cells were infected with SARS-CoV-2 (MOI 1) for 24h. The cells were hybridised with smFISH probes targeting ORF1a of positive and negative genomic RNAs. The number of viral RNAs in a replication centre (yellow circle) were resolved by fitting the point-spread function of a single RNA molecule in 3D volume and are stated in the figure. ~95% detection efficiency was achieved (n = 5).*

This project will be co-supervised by Jane McKeating, Timothy Hinks and Ilan Davis who provide complementary expertise in molecular virology, respiratory immunology and mRNA biology, respectively. The project provides a unique training environment to apply super resolution imaging techniques to visualize replicating viral RNAs in complex tissues. A range of techniques will be offered and the student will become proficient with SARS-CoV-2 infection and replication models, primary bronchial epithelial cell culture systems, design and validation of probes for single molecule fluorescent in situ hybridization and down-stream analytical skills. Transferable skills including oral presentations at joint lab meetings, critical reviewing of published scientific literature by contributing to journal clubs and scientific writing by reviewing and drafting manuscripts for publication.

Supervisors

Jane McKeating graduated from their DPhil studies at University College, London in 1987 and has worked on clinical important viral pathogens including HIV, hepatitis C virus and more recently HBV. The main interest of her laboratory is to understand the role of hypoxia and circadian signaling pathways to regulate different steps in virus replicative life cycle. Her

laboratory has identified many of the receptors defining hepatitis C virus entry into the liver and pathways for viral dissemination. Jane has published over 170 research papers on HIV, hepatitis B and C viruses and that have received over 19,500 citations (Current H-index of 71 – SCI) and serves on the editorial board of several journals and Wellcome Trust Infection and Immunity Expert Review group.

https://www.ndmrb.ox.ac.uk/team/jane-mckeating;
https://www.ndm.ox.ac.uk/principal-investigators/researcher/jane-mckeating

Timothy Hinks is a Wellcome Trust Career Development Fellow. His group research the immunology of airways diseases. As a Consultant in Respiratory Medicine he co-leads the Oxford Specialist Airways service at the John Radcliffe Hospital in Oxford. Timothy studied medicine at Cambridge and Oxford where he studied T cell immunodiagnostics for tuberculosis. He since undertook a Wellcome-funded PhD in Southampton investigating the roles of innate and adaptive T cells in the human airways in asthma, and a Wellcome Postdoctoral Fellowship with James McCluskey at the Peter Doherty Institute, University of Melbourne researching the basic biology of mucosal associated invariant T cells in bacterial and viral respiratory infections and in tissue repair. His group's current research focuses are defining the innate-like mucosal T cell response to bacterial infection in airways disease, investigating the immune mechanisms underlying macrolide efficacy in asthma, and running the ATOMIC2 trial in COVID-19. His group investigatesthe genetics, transcriptomics and epigenetics of severe asthma and COVID-19.

https://www.ndm.ox.ac.uk/team/timothy-hinks

Ilan Davis graduated from their DPhil at Imperial Cancer Research Fund's Developmental Biology unit in Oxford, where he pioneered the discovery that mRNA localizes according to small signals in their 3'UTRs during pattern formation. During his postdoctoral studies at UCSF in San Francisco, Ilan pioneered the use of GFP to study cell division in living embryos. When he first established his own group in Edinburgh as a Lister and Wellcome Fellow, he developed novel technologies for live cell imaging of mRNA that enabled seminal discoveries; dynein motors transport mRNA along microtubules and statically anchor them at their final destination; processing bodies triage mRNAs for internal translational repression versus activation. Their current research program involves understanding how biological processes, including viral infection, are controlled at the post-transcriptional level by mRNA binding proteins, mechanisms that are as important as transcriptional activation. Ilan was elected as an EMBO fellow in 2010, has published 94 papers and serves on the scientific advisory board of a "big-data" technology startup and the OMERO-IDR project, editorial board of two journals and on the UKRI-MRC molecular medicine panel.

http://www.ilandavis.com/home.html
https://www.bioch.ox.ac.uk/research/davis
https://micronoxford.com/about-us

Key publications:

1. Kounatidis I, Stanifer ML, Phillips MA, Paul-Gilloteaux P, Heiligenstein X, Wang H, Okolo CA, Fish TM, Spink MC, Stuart DI, Davis I, Boulant S, Grimes JM, Dobbie IM, Harkiolaki M. (2020) Correlative cryo-structured illumination fluorescence microscopy and soft X-ray tomography elucidates reovirus intracellular release pathway. Cell 182: 515–530.e17. pdf

2. Zhuang X, Magri A, Lai AG, Hill M, Chang WH, *et al*. 2019. The circadian clock BMAL1 and REV-ERB regulate flavivirus replication. Nature Comms **10**:377. pdf

3. Garcia-Moreno M, Noerenberg M, Ni S, Järvelin AI, González-Almela E, Lenz CE, Bach-Pages M, Cox V, Avolio R, Davis T, Hester S, Sohier TJM, Li B, Heikel G, Michlewski G, Sanz MA, Carrasco L, Ricci EP, Pelechano V, Davis I, Fischer B, Mohammed S, Castello A. (2019). System-wide Profiling of RNA-Binding Proteins Uncovers Key Regulators of Virus Infection. Molecular Cell 74: 196-211.e11. pdf

4. van Wilgenburg B, Loh L, Chen Z, Pediongco TJ, Wang H, Shi M, Zhao Z, Koutsakos M, Nüssing S, Sant S, Wang Z, D'Souza, C, Almeida CF, Kostenko L, Eckle SBG, Meehan BS, Godfrey DI, Reading PC, Corbett AJ, McCluskey J, Klenerman P, Kedzierska K, Hinks TSC MAIT cells contribute to protection against lethal influenza infection in vivo *Nature Commun* 2018 Aug 22;9(1):3350. pdf

5. Hedegaard DL, Tully DC, Rowe IA, Reynolds GM, Hu K, *et al*. 2017. High resolution sequencing of hepatitis C virus reveals limited intra-hepatic compartmentalization during late stage liver disease. J Hepatology **66**: 28-38. pdf

6. Mailly L, Leboeuf C, Xiao F, Lupberger J, Wilson GK, *et al*. 2015. Clearance of persistent hepatitis C virus infection using a monoclonal antibody specific for tight junction protein Claudin-1. Nature Biotechnology **33**: 549-54. pdf

7. Kostenko L, Turner SJ, Corbett, AJ, Chen Z, Klenerman P, McCLuskey J Activation and in vivo evolution of the MAIT cell transcriptome in mice and humans reveals diverse functionality *Cell Reports* 2019 Sep 17;28(12):3249-3262.e5. pdf

Project 11

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

Supervisor: Professor Peijun Zhang - peijun@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, part of our knowledge is largely based on the previous coronaviruses, very little is known about 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 determinants of SARS-CoV-2 replication and their molecular architectures, 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 sub-nanometer resolution or better using 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 coronaviruses.

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 viral infection
- ✓ Data analysis and image reconstruction
- ✓ Computer simulations

Supervisor

Professor Peijun Zhang obtained her Ph.D. in Biophysics and Physiology from University Virginia, M.S. in Physics and B.S. in Electrical Engineering from Nanjing University, China. She was a postdoc and subsequently a staff scientist at the National Cancer Institute, NIH. In 2006, she joined the faculty of the University of Pittsburgh, and was promoted to associate professor with tenure in 2012. In 2016, she joined the University of Oxford as a full professor,

and jointly as the founding director of eBIC (the UK National CryoEM Facility) at the Diamond Light Source. Her research focuses on the molecular mechanisms of host and pathogen interactions, including SARS-CoV-2, HIV-1 and pathogenic bacteria, by developing and combining novel technologies for high-resolution cryoEM and cryoET. She received many awards, including "Carnegie Science Emerging Female Scientist Award" (2014) and "Wellcome Trust Investigator Award" (2017). She has supervised 27 Postdocs, 7 PhDs, 2 Masters and 8 Undergraduate students.

Key publications

1. 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.

2. Himes B.A. and **Zhang P.*** (2018) emClarity: Software for High Resolution Cryo-electron Tomography and Sub-tomogram Averaging. *Nat Methods* 15(11):955-961

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. Sutton G, Sun D, Fu X, Kotecha A, Hecksel CW, Clare DK, **Zhang P*,** Stuart DI*****, Boyce M***** (2020) Assembly intermediates of orthoreovirus captured in the cell. *Nat Commun* **11,** 4445.

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

Project 12

Project Title: Iron and the anti-tumour immune response

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

Project Overview

Activating host T-cell responses against tumours can significantly improve the outcome of a number of cancers. To be effective, these antigen-specific lymphocyte responses have to be large, systemic, functional, and long-lived. Understanding the factors that can limit anti-tumour immunity is important to guide therapy and to design better protocols for improving patient responses. Children with a rare mutation impairing cellular iron uptake have profoundly impaired immunity. In mice we have established that the size and efficacy of immune responses to a range of vaccines and to influenza virus infection is controlled by the amount of iron that is available to the responding lymphocytes. In humans with melanoma undergoing checkpoint inhibitor therapy, we found that checkpoint inhibitor therapy led to transcriptional changes in responding CD8+ T cells that enable efficient iron acquisition. However, iron deficiency and anaemia are common in cancer patients, and so iron availability to lymphocytes is likely to be frequently suboptimal at a time when the anti-tumour immune response needs iron to develop. We propose a study to establish whether the immune responses to tumours, induced either by vaccination or by checkpoint inhibitor therapy, can be hampered by iron deficiency, and in particular whether responses can be boosted by increasing iron availability. The latter will be achieved by suppressing activity of the iron regulatory hormone hepcidin, and we will test a variety of methods to do this, many of which we have developed ourselves. Parallel work with human samples will assess whether iron deficiency in cancer patients influences outcome of checkpoint inhibitor therapy and will investigate transcriptional signatures of iron deficiency in lymphocytes responding to therapy.

Training opportunities

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

Supervisor

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

Clinical supervisors

Ben Fairfax (https://www.imm.ox.ac.uk/people/benjamin-fairfax)

We will also receive advice from Paul Klenerman (https://www.ndm.ox.ac.uk/team/paul-klenerman)

Key publications

1. Arezes JA et al. Erythroferrone inhibits the induction of hepcidin by BMP6. Blood. 2018 132:1473-14

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

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

4. Frost JN et al, Hepcidin-mediated hypoferremia disrupts immune responses to vaccination and infection. Under review.

Project 13

Project Title: Dissecting antiviral T cell responses to SARS-CoV-2 in the setting of HIV infection

Supervisor: Professor Sarah Rowland-Jones - sarah.rowland-jones@ndm.ox.ac.uk

Project Overview

The global outbreak of SARS-CoV-2 has reportedly resulted in an overall 3% case fatality rate. Increased mortality and morbidity have been reported in immunocompromised individuals, and is seen with advancing age. It is thought that hyper-inflammation is a major driver of severe illness with COVID-19. Perhaps surprisingly, HIV-infected people on effective antiretroviral therapy (ART) and with a good CD4 count are not deemed to be at increased risk of COVID-19 complications. Nevertheless, people living with HIV (PLWH) experience significant co-morbidity associated with premature aging and persistent immune dysfunction and immune activation despite effective ART. With the number of older PLWH (>50 years) predicted to increase by 47% to 6.9 million in 2020 more research is necessary to understand the relationship between HIV and SARS-CoV-2 infection. At present there is a dearth of immunological studies, exploring the intersection between HIV infection and the novel coronavirus, including data on long-lasting T cell immunity to SARS-Cov-2 and whether these responses may be compromised in HIV-infected individuals relative to HIV negative donors, particularly in the context of persistent HIV-related immune activation.

The **premise** of this project is therefore to investigate SARS-CoV2 specific immunity in PLWH compared to HIV negative individuals with a history of COVID-19 infection. **The main aim** is to characterise virus-specific T-cell immune responses to SARS-CoV2 longitudinally in the presence or absence of HIV infection. This study will provide extremely pertinent information that will broaden our understanding of host-pathogen interactions following infection with the novel coronavirus in PLWH and HIV negative individuals, and in turn inform how to optimally manage and risk-stratify HIV-infected individuals during the next waves of COVID-19. The identification of correlates of immune protection and immune differences in the setting of pre-existing inflammatory conditions (such as HIV) will better define therapeutic strategies and new targets for vaccine development for COVID-19.

Training opportunities

This study will build on our extensive combined research expertise in studies of T-cell immunity in both acute and chronic viral infection, and benefits from a well-curated cohort of patient samples forming part of our prospective longitudinal study. Analysis of immune responses will be done at extraordinary resolution, using unbiased single cell RNA sequencing (sc-RNAseq), combined with MHC class I/II multimer technology, phenotypic and functional T-cell assays. The successful candidate will gain superb training in T cell immunology, including advanced single cell (sc)-RNA sequencing genomic analysis and bioinformatic pipelines in combination with advanced conventional immune cell functional and phenotypic analysis in flow cytometric assays.

Supervisors

Professor Sarah Rowland-Jones and Dr Dimitra Peppa are both clinician scientists with honorary consultant contracts in adult Infectious Diseases (SR-J) and Sexual Health/HIV

medicine (DP). SR-J has several decades of experience in studying host-pathogen interactions in HIV cohorts from around the world. DP has pioneered NK and T-cell studies in persistent infection with HIV and HBV, and has established the cohort in which these samples have been collected. Professor Tao Dong (CAMS PI) has recently led detailed studies of T-cell responses in SARS CoV-2 infection, against a background of extensive experience in cellular immunology in HIV and HBV infection and malignancies.

Key publications

1. Bradley T, Peppa D, Pedroza-Pacheco I, Li D, Cain DW, Henao R, Venkat V, Hora B, Chen Y, Vandergrift NA, Overman RG, Edwards RW, Woods CW, Tomaras GD, Ferrari G, Ginsburg GS, Connors M, Cohen MS, Moody MA, Borrow P, Haynes BF. RAB11FIP5 Expression and Altered Natural Killer Cell Function Are Associated with Induction of HIV Broadly Neutralizing Antibody Responses. Cell. 2018;175(2):387-399 e317.

2. de Silva TI, Peng Y, Leligdowicz A, Zaidi I, Li L, Griffin H, Blais ME, Vincent T, Saraiva M, Yindom LM, van Tienen C, Easterbrook P, Jaye A, Whittle H, Dong T, Rowland-Jones SL. Correlates of T-cell-mediated viral control and phenotype of CD8(+) T cells in HIV-2, a naturally contained human retroviral infection. Blood. 2013;121(21):4330-4339.

3. Peng Y, Mentzer AJ, Liu G, Yao X, Yin Z, Dong D, Dejnirattisai W, Rostron T, Supasa P, Liu C, Lopez-Camacho C, Slon-Campos J, Zhao Y, Stuart DI, Paesen GC, Grimes JM, Antson AA, Bayfield OW, Hawkins D, Ker DS, Wang B, Turtle L, Subramaniam K, Thomson P, Zhang P, Dold C, Ratcliff J, Simmonds P, de Silva T, Sopp P, Wellington D, Rajapaksa U, Chen YL, Salio M, Napolitani G, Paes W, Borrow P, Kessler BM, Fry JW, Schwabe NF, Semple MG, Baillie JK, Moore SC, Openshaw PJM, Ansari MA, Dunachie S, Barnes E, Frater J, Kerr G, Goulder P, Lockett T, Levin R, Zhang Y, Jing R, Ho LP, Oxford Immunology Network Covid-19 Response TcC, Investigators IC, Cornall RJ, Conlon CP, Klenerman P, Screaton GR, Mongkolsapaya J, McMichael A, Knight JC, Ogg G, Dong T. Broad and strong memory CD4(+) and CD8(+) T cells induced by SARS-CoV-2 in UK convalescent individuals following COVID-19. Nat Immunol. 2020.

4. Peppa D, Gill US, Reynolds G, Easom NJ, Pallett LJ, Schurich A, Micco L, Nebbia G, Singh HD, Adams DH, Kennedy PT, Maini MK. Up-regulation of a death receptor renders antiviral T cells susceptible to NK cell-mediated deletion. J Exp Med. 2013;210(1):99-114.

5. Yindom LM, Simms V, Majonga ED, McHugh G, Dauya E, Bandason T, Vincon H, Rylance J, Munyati S, Ferrand RA, Rowland-Jones SL. Unexpectedly High Prevalence of Cytomegalovirus DNAemia in Older Children and Adolescents With Perinatally Acquired Human Immunodeficiency Virus Infection. Clin Infect Dis. 2019;69(4):580-587.

Project 14

Project Title: The Transcriptomic and inflammatory phenotype of non-specific pleuritis

Supervisor: Professor Najib M Rahman - najib.rahman@ndm.ox.ac.uk

Background

Pleural effusion is the accumulation of fluid in the pleural cavity, the area between the lungs and the chest wall. A pleural effusion may be attributed to many different medical aetiologies malignant or benign. The identification of the aetiology is important for successful management.

Non-specific pleuritis (NSP) is defined as fibrinous or inflammatory pleuritis which cannot be attributed to a specific benign or malignant aetiology. It can be diagnosed in biopsies in up to one third of cases. As a result, patients are followed up for one to two years. Approximately 15% of patients are later diagnosed with a malignant pleural disease mostly mesothelioma, which is the primary malignancy of the pleural space.

The inflammatory profile of NSP remains unknown. Moreover, there are not available biomarkers to guide diagnosis or treatment. The understanding of the underlying nature and biology of NSP is of paramount importance for the clinical management of these patients. Furthermore, this could lead to less invasive procedures and reduce the healthcare cost.

Project overview

We have access to a unique biobank of pleural fluid, pleural biopsies and blood biospecimens from patients with pleural effusion from various aetiologies. We have designed a translational project to thoroughly study the transcriptomic and inflammatory profile of NSP. Pleural fluid samples from NSP and non-NSP patients will be subjected to RNA-Sequencing. The comparison between different aetiologies will elucidate the differences at the transcriptomic level. The unique signature of NSP will be used to phenotype the inflammatory panel of NSP.

The aim of the study is to characterize the distinct transcriptomic and inflammatory phenotype of NSP and discover biomarkers that could guide diagnosis and treatment.

Training opportunities

The proposed project is a collaboration between the Chinese Academy of Medical Sciences-Oxford International, the Oxford Respiratory Trials Unit, the Centre for Translational Immunology and the Laboratory of Translational Pleural Research. The PhD student, ideally a medical graduate, will collaborate with leading clinicians and scientists in pleural and immunology research. The strength of the proposed translational project relies on the close collaboration and interaction between the clinic and the laboratory. To this end, the student will obtain valuable clinical experience in pleural procedures (pleural biopsy, ultrasound guided thoracentesis) and laboratory techniques including cell culture, Fluorescence Activated Cell Sorting (FACS) and molecular biology techniques. This is a project with the potential to lead to a clinical trial, such that the student will have the opportunity to be

involved in clinical trial design and implementation, using expertise in methodology from the Trials Unit.

Supervisor:
Professor Najib M Rahman

Qualifications:
DPhil: Clinical Medicine, University of Oxford, 2010
MSc: Clinical Trials, London School of Hygiene and Tropical Medicine, 2009

Current Positions:
Director of the Oxford Respiratory Trials Unit, Consultant and Lead for Pleural Disease in Oxford, Professor of Respiratory Medicine

Research Area:
Clinical studies in malignant and infectious pleural disease

Ongoing Research:

1. A prospective multinational cohort study of risk factors, microbiology and outcome in pleural infection.
2. Prospective randomised assessment of the use of thoracic ultrasound in pleurodesis prediction for malignant effusion.
3. Three randomised multi-centre studies assessing optimal analgesia and drain size, the clinical value of indwelling pleural catheters and intrapleural fibrinolytic agents in the treatment of malignant pleural effusion.
4. The pathogen identification in pneumonia and pleural infection study, assessing molecular and novel radiological diagnostic techniques in thoracic infection.
5. A definitive phase III study assessing intrapleural tPA and DNase for the treatment of pleural infection (MIST3).
6. Detailed radiological description of pleural infection, and its correlates to microbiology and outcome.
7. Development work using a number of novel biological agents to manipulate pleural fluid production / pleurodesis.

CAMS PI – Co-supervisor:
Professor Tao Dong

Tao Dong has held the post of Professor of Immunology in the MRC Human Immunology Unit at Oxford University since 2014 and is a Senior Fellow at University College Oxford. She has served as a member of the UK Medical Research Council Infection and Immunity board between 2016-2020. She is founding director of CAMS Oxford joint international Centre for Translational Immunology since 2013, and founding director (Oxford) of CAMS Oxford Institute based in Nuffield Department of Medicine, Oxford University since 2019. Tao originally gained a BSc degree in Physiology from Fudan University, Shanghai, China in 1987. She moved to Oxford University in 1993 where she received a DPhil degree in Immunology in 1998 for work carried out under the supervision of Professors Sarah Rowland-Jones and

Sir Andrew McMichael on qualitative changes in HIV-specific cytotoxic T cells associated with HIV disease progression. During her postdoctoral training, where she continued to study immune responses to HIV, she expanded her research interests to include work on influenza virus infection, which led her to start her own independent research group. In 2010 she became the Head of the human anti-viral and anti-cancer cytotoxic T cell laboratory and a Program Leader in the MRC Human Immunology Unit at Oxford University. Since 2013, the main focus of her research has switched from virus infections to cancer, with a central goal being to identify determinants of the ability of human tumour-specific cytotoxic T cells to control human tumour development and metastasis.

Co-supervisor:
Dr. Nikolaos Kanellakis

Highly motivated, hardworking and team-player. Having a keen scientific interest into pleural diseases and malignancies translational research with five years of experience and relevant publications. I am an active member of European Respiratory Society and British Thoracic Society.

Current posts:
1. Lead Postdoctoral Research Fellow, Laboratory of Pleural and Lung Cancer Translational Research, Oxford Respiratory Trials Unit, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom.
2. Teaching Lecturer in Medicine, University College, University of Oxford, United Kingdom.

Qualifications:
1. Doctor of Philosophy, Medical Faculty. University of Patras, Greece. Graduated with honours. PhD thesis title: “Mouse tobacco carcinogen induced lung adenocarcinoma cell lines as tools to identify novel lung cancer genes”. Graduated with honours.
2. Master of Science: Informatics of Life Sciences, Medical Faculty, University of Patras, Greece. Master thesis title: “The role of Geminin in embryonic Neural Crest Cells a functional approach through bioinformatics analyses”. Graduated with honours.
3. Bachelor, Computer Engineering & Informatics Department, School of Technology, University of Patras, Greece. Graduated with honours.

Fellowships – Prizes: European Respiratory Society Short Term Research Fellowship, Oxford Centre for Respiratory Medicine, Churchill Hospital, Oxford, UK, March 2016
Award from the Computer Engineering and Informatics Department for the best undergraduate student performance, Top 5% of class 2012, July 2012

Key Publications

1. Enriched HLA-E and CD94/NKG2A Interaction Limits Antitumor CD8+ Tumor-Infiltrating T Lymphocyte Responses. *Cancer Immunol Res*. 2019

2. Development and validation of response markers to predict survival and pleurodesis success in patients with malignant pleural effusion (PROMISE): a multicohort analysis. *Lancet Oncol.* 2018

3. Activated innate lymphoid cell populations accumulate in human tumour tissues. *BMC Cancer*. 2018

4. Effect of Opioids vs NSAIDs and Larger vs Smaller Chest Tube Size on Pain Control and Pleurodesis Efficacy Among Patients With Malignant Pleural Effusion: The TIME1 Randomized Clinical Trial. *JAMA* 2015

5. Effect of an indwelling pleural catheter vs chest tube and talc pleurodesis for relieving dyspnea in patients with malignant pleural effusion: the TIME2 randomized controlled trial.
6. *JAMA*. 2012

7. Outcome of patients with nonspecific pleuritis/fibrosis on thoracoscopic pleural biopsies, European Journal of Cardio-Thoracic Surgery, Volume 38, Issue 4, October 2010, Pages 472–477, https://doi.org/10.1016/j.ejcts.2010.01.057

Project 15

Project Title: Understanding Correlates of Protection for SARS-CoV-2: Studying naturally acquired and vaccine induced immunity in humans and non-human primates (NHPs)

Supervisor: Professor Miles W Carrol - Miles.Carroll@phe.gov.uk

Project Overview

Correlates of protection (CoP) are defined as specific immune response components that are strongly associated with protective immunity or improved clinical outcome after pathogen exposure. Knowledge of CoP can enhance the design of efficacious vaccines and, when validated, greatly accelerate vaccine licensure. Correlates have previously been identified as serum based that possess unique antigen binding and immune functions. They have also been shown to be general T helper type as is the case for pertussis. Over the last 4 years our group has been studying naturally acquired immunity to Ebola virus and comparing it to vaccine induced immunity. Our studies suggest that immunoglobulin Fc function may be involved in enhanced viral neutralisation, additionally we have shown a specific poly-functional T cell phenotype is also associated with protective immunity (Thom et al 2020 Lancet ID).

More recently we have performed vaccination and challenge studies, using 4 candidate vaccines, in an established COVID-19 rhesus macaque challenge model (Salguero et al 2020 Nature Comms in review). Using this unique sample set we will further our understanding of those innate and adaptive immune mechanisms that result in reduced lung pathology and virus load post challenge with SARS-CoV-2. We hypothesise that both humoral and cellular mechanisms contribute to protection. The student will apply a range of methods including Ig Fc functional assessment i.e. complement deposition and neutralisation enhancement, in addition to poly functional phenotype analysis. Working with colleagues across the Nuffield Department of Medicine (NDM), comparisons with naturally acquired immunity in a human COVID convalescent cohort can be made. Linking the immune characterisation with the array of NHP pathology and virology parameters via a network analysis will reveal the relationship between immunity and protection.

Training opportunities

The student will join an established viral immunology laboratory within the NDM that has all the skills necessary to undertake the research to characterise immunity to SARS-CoV-2 and identify potential correlates of protection. The team have a successful track record of training PhD students and ensure they have an opportunity to draft and author good quality research papers published in high impact factor journals. The student will be trained in an array of immune based laboratory skills including flow cell analysis, T Cell activation, antibody characterisation and complement assays. Platforms such as transcriptomics and associated bioinformatics may also be utilised. The PI (Miles Carroll) has a joint appointment at the UK Department of Health research facility at Porton Down where the in vivo studies have been performed. This offers an opportunity for the student to carry out a proportion of their studies in another research establishment and learn about the many ongoing emerging disease research projects.

Supervisor

Miles Carroll has over 25 years of experience in virology, immunology and vaccine research. After completing his post-doctoral training at the National Institutes of Health in the USA he spent 12 years in the biotech sector working on the development of cancer immunotherapy vaccines and anti-virals. Since 2008 Miles has been Head of Research at PHE Porton Down. During the 2013-2016 west African Ebola virus outbreak he played a key role in the introduction of real time sequencing in support of molecular epidemiology (Carroll Nature 2015 & Quick Nature 2016). In the field he supported the successful EBOV vaccine phase III efficacy study (Lancet 2015/16). His group has just completed a 4 year longitudinal study to characterise immunity in Ebola virus disease survivors (Thom at al 2020 Lancet ID & Tom et al 2020 Nature Comms In Press). Since January 2020 Prof Carroll has dedicated his research to the COVID-19 response. He has concentrated his efforts on the establishment of in vivo models to assess the efficacy of COVID-19 vaccines and therapeutics. Since January 2020 he has authored 25 COVID-19 research papers in a variety of journals and pre-print platforms.

Co-supervisor: Professor Tao Dong is Professor of Immunology, MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine. Director(Oxford), CAMS-Oxford Institute, Nuffield Department of Medicine, Oxford University. Tao has an international reputation for her T cell immunology studies primarily based on influenza and more recently SARS-CoV-2.

Key publications

1. Thom R …… & Carroll MW 2020 Longitudinal antibody and T cell responses in Ebola virus disease survivors and contacts: an observational cohort study. Lancet Infectious Diseases In Press

2. Salguero FJ…& Carroll MW 2020 Comparison of Rhesus and Cynomolgus macaques as an authentic model for COVID-19 BioRxiv: In review Nature Comms

3. Timothy J….& Carroll MW 2019 Early transmission and case fatality of Ebola virus at the index site of the 2013–16 west African Ebola outbreak: a cross-sectional seroprevalence survey. Lancet Inf. Dis 19 429-438.

4. Davis C, Tipton T, ……..Miles W Carroll & Emma Thomson 2019 Post-exposure prophylaxis with rVSV-ZEBOV following exposure to a patient with Ebola virus disease relapse in the UK: an operational, safety and immunogenicity report. Clin Inf Dis

5. Quick J, Loman N…. & Carroll MW 2016 Real-time, portable genome sequencing for Ebola surveillance Nature 530 228-232.

Project 16-1

Project Title: The epitope abundance-avidity-efficacy axis in cancer

Supervisor: Prof Tim Elliott - T.J.Elliott@soton.ac.uk

Collaborators
Prof Mark Middleton (NDO) – Medical Oncology, experimental clinical trials
Prof Xin Lu (Ludwig Institute) – Cancer biology, deep phenotyping
Prof Tao Dong (NDM) – Cancer Immunology, Cytotoxic T cell function, epitope detection

Introduction
The infiltration of tumours with CD8+ T cells (particularly CD103+ Resident memory CD8+ T cells) correlates with better prognosis (1) and a positive outcome in checkpoint blockade immunotherapy (2); and correlates with a tumour gene signature in which the antigen processing and presentation module is upregulated (3). Furthermore, loss of expression of APM genes frequently correlates with poor outcome (4-6); and loss of MHC I heterozygosity during tumour evolution is a marker of poor overall survival (7). Consequently, epitopes targeted by CTL in tumours is currently a subject of fierce interest. Neoantigens, ie epitopes encoded by tumour-specific mutations (or tumour specific post-translational peptide modification) are emerging as crucial targets and although there is some correlation between the mutational burden of a tumour (and therefore the theoretical number of neoepitopes), this is insufficient to explain differences in tumour infiltration with CD8+ CTL and other markers of effective CD8+ T cell mediated immune control []. New paradigms are emerging aimed at understanding (and predicting) the likelihood of specific neoepitopes prompting effective antitumour CTL responses in immunotherapeutic settings such as checkpoint blockade therapy CBT and therapeutic vaccination. These include peptide affinity, homology to microbial peptides and probability scores for TcR recognition. Factors relating to the antigen-processing pathway are also important and include the source and abundance of translated products that enter the processing pathway [], processing enzymes including highly polymorphic ERAP1, competition between peptides during the selection process, and the action of tapasin, which varies depending on HLA type of the patient. Together, these control the relative abundance of different peptide:MHC complexes at the cell surface [2, 5] which in turn determines the hierarchy of CTL responses in vivo [5]; and possibly the quality of CTL response that is elicited: that is to say the generation of CTL that are more likely to improve outcomes following CBT and therapeutic vaccination.

Background
We have shown that the antigen presentation machinery regulates antigen abundance at steady state on the surface of cells and that this correlates with immunodominance in a DNA vaccine, viral infection and tumour setting (57-59). In the latter case, we increased tumour immunogenicity by increasing the abundance of an epitope that is regulated by ERAP1 (59).
There are many examples in the literature that indicate an inverse relationship between antigen dose and T cell avidity (eg. (19, 20), including peptide-vaccine studies in cancer patients (60). We have shown that peptide-specific T cells primed to recombinant virus in mice that lack tapasin – where epitope abundance is significantly reduced, have a functional

avidity two orders of magnitude higher than T cells primed in tapasin-competent mice where epitope abundance is much higher (58).

Though the superiority of high avidity T cells in infections and cancer is often asserted (61-63), other studies point to the relevance of low-avidity T cells in controlling chronic virus infections and established tumours (22-25). Indeed, low-avidity T cells might (a) better distinguish between tumours overexpressing self-antigens and healthy self-tissue (24), (b) be less sensitive to checkpoint regulation, activation-induced cell death (63, 64), senescence and exhaustion, leading to protracted survival of functionally-competent T cells, and (c) be less likely to induce tumour escape (23, 65, 66).

In the CT26 tumour model where the immunodominant epitope GSW11 is highly abundant, we observe the priming of a diverse population of GSW11-specific CD8+ T cells which are suppressed or exhausted in the tumour microenvironment (31). These T cells have a range of avidities, yet the low avidity clones are more readily suppressed by Treg and their expansion (in response to either Treg depletion or immune checkpoint blockade with anti-PD1) correlates with protection in immunotherapy experiments (32). Moreover, we have found that the immunophenotype of this population, in contrast to high avidity CD8+ T cells recognising the same peptide, resembles that of precursor exhausted T cells seen in chronic viral infection and cancer (67). Importantly, this population has been shown to be responsive to reinvigoration by anti-PD1 CBT, unlike its terminally differentiated counterpart.

Taken together with recent models of epitope fitness – based on their homology to microbial peptides, we have investigated the quality of CTL responses generated in a syngeneic mouse tumour model (CT26) where some target epitopes are derived from an endogenous retroviral glycoprotein that is not expressed in neonatal thymus and therefore superficially resembles a neoepitope with a high quality score.

One of these epitopes (GSW11) is particularly interesting because although it has a very low affinity, it is abundant at the cell surface and is especially sensitive to editing in the antigen processing pathway. Previously, we have shown that epitope abundance controls immunodominance for several non-cancer epitopes in two vaccination settings, and we have also shown that the (tumour) cell surface abundance of GSW11 controls its immunogenicity in the CT26 model. GSW11 is sensitive to tapasin editing, and its abundance at the cell surface increases when ERAP1 is inhibited leading to its enhanced immunogenicity. Low affinity CTL recognising this peptide are preferentially suppressed by Treg and can be re-activated upon Treg depletion where they become therapeutically highly effective (**Sugyarto et al 2020, *in press***). De-suppression of the same CTL also correlates with therapeutic efficacy in anti-PD1 immunotherapy. We have shown that these CTL have a partially exhausted phenotype similar to those described in chronic viral infection and PD1-responsive human melanoma (**Sugyarto et al, *in preparation***). Taken together, these data suggest that an inverse correlation may exist between the avidity of antitumour CTL and their quality, and furthermore that the generation of low avidity CTL may correlate with a high abundance of epitope presented at the tumour cell surface – a parameter that is ultimately under control of the antigen processing pathway.

Proposal

3.1 Proof of immunological concepts in mouse models.

- *The relationship between antigen dose, T cell avidity and function in the CT26 model:*

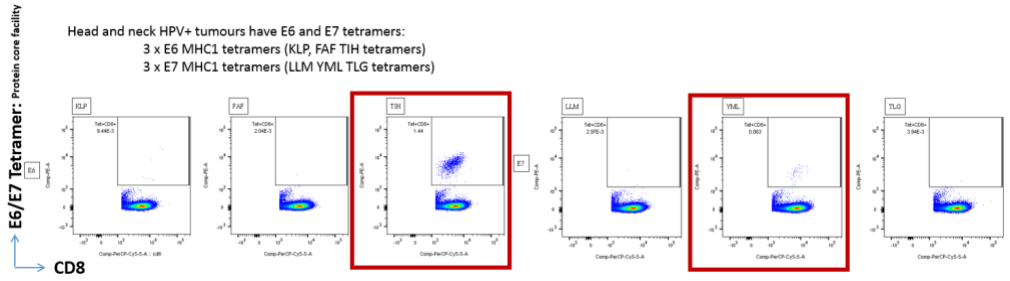
We have shown that it is possible to enhance the immunogenicity of CT26 by increasing the abundance of the GSW11:D^d complex as a SCT transgene. To test the relationship between p:MHC abundance, immunogenicity, CTL avidity and response to CBT, we will generate a panel of CT26gp90-/- transfectants expressing graded levels of the GSW11:D^d SCT. These will be correlated to tumour growth, TIL specificity, avidity of anti-GSW11 T cells expanded in response to CBT. We have also shown that it is possible to increase the abundance of GSW11:D^d by manipulating ERAP1 expression, and this leads to better immunogenicity. We will isolate TIL from regressing CT26ERAP1-/- tumours and enumerate high and low avidity (GSW11-specific) TIL, using both tetramer run-off experiments and a new technical platform: the Lumicks Movi-Z cell interaction platform which is capable of measuring the force (in pico Newtons) required to separate T cells from their targets using an ultrasound forcefield.

- *The relationship between antigen dose and CD8+ T cell avidity in an anticancer cDNA vaccine setting:* Our observation may be relevant to anti-cancer vaccination, where a significant barrier is the tumour-induced exhaustion (or inactivation) of vaccine-induced T cells (69). We have shown that it is possible to manipulate immunodominance to competing SV40T epitopes delivered as DNA fusion vaccines by altering p:MHC abundance via peptide affinity (70). We will evaluate the response (relative abundance of the 4 specificities in TIL, and their avidity) of tumour-bearing mice to vaccination with constructs delivering different abundancies of dominant and subdominant epitope. Experiments will be performed initially in B6 mice transplanted with the prostate cancer SV40T-expressing TRAMP-C1 line, then the TRAMP GMM, which uniformly and spontaneously develop prostate tumours driven by SV40T.

We will evaluate responses (specificity and avidity) to anti-PD1 CBT in the 40-50% of vaccinated mice in which we observe progressing tumours by isolating low and high avidity CD8+ anti- SV40T TIL at the point where response is evident, using tetramers, quantifying T cells, and characterising their immunophenotype.

3.2 Low avidity CTL in human cancer

Figure: Proof of concept that high and low avidity anti E6/E7 CD8+ T cells can be identified among TIL of an HNSCC patient



- In HNSCC, we have observed the presence of low avidity oligoclones recognising the well-characterised HPV-1 E6/7 derived HLA-A*0201 restricted LV9 epitope (low tetramer staining example in Figure below). The Lumicks platform offers the possibility of measuring average T-cell avidities for oligoclonal TIL of unknown specificity, and avidity-sorting them. We are currently validating the platform using the tetramer-sorted cell populations described above and autologous tumour target

cells. As part of this project, we will isolate high and low-avidity T cells recovered from TIL as they become available from patient biopsies and/or resected tumours. Where possible, we will integrate this analysis with an ongoing investigation into the correlates of response to combined anti-PD-L1 in combination with chemo-radiotherapy for gastro-oesophogeal cancer (the LUD2015 005 trial in collaboration with Profs Mark Middleton (Oncology) and Xin Lu (Ludwig Institute)). Avidity-sorted CD8+ T cells will be processed for bulk RNAseq to look for different gene signatures, particularly those correlating with precursor vs terminal exhaustion phenotypes, which have been observed by others in TIL (71). We will relate these data to longitudinal clinical data to determine whether there is a correlation between the expansion of low avidity T cells and tumour regression.

Training Opportunities

This project is suitable for students who are interested in combining quantitative biological approaches with preclinical modelling as a route to investigating human immune responses to cancer. A wide variety of lab techniques will be used and you will be become expert in cellular immunology and molecular biology as well as in using mouse models to investigate complex immunological responses. Students will be registered on a techniques course in the first year which covers the latest techniques in cellular and molecular analysis, including the very latest experimental platforms. Taught modules in immunology are available in the university, as is professional training in animal experimentation. You will also be trained in the handling, processing and governance of human clinical samples and the principles of experimental medicine. Students will attend and participate in relevant seminar programmes within the NDM, WIMM , Ludwig Institute and NDO which provide the opportunity to meet and learn from international leaders in your field and build an exceptional academic network of contacts. Other specialist courses are available in Oxford and at other institutions in the UK, as necessary.

Supervisor Profile

Tim Elliott was amongst the key group of immunologists who developed the field of antigen presentation at the molecular level during the 1980s, undertaking a series of studies to determine and define the immunostimulatory properties of MHC Class I molecules and elucidating the molecular mechanisms of co-factor assisted peptide loading of MHC Class I in antigen presenting cells: work considered to be the fundamental foundation of much of the recent work on antigen presentation. The work underpins rational T-cell based vaccine design and continues to fuel translational research where discoveries in the areas of antigen discovery, T cell regulation and immunodominance are making a significant impact on new and ongoing cancer immunotherapy trials. His mechanistic studies have always benefitted from an active interface with the physical sciences including collaborations with synthetic and computational chemistry, computer science, mathematics and engineering. He is the founding Kidani Chair of Immune-oncology, a Fellow of the Royal Society for Biology and the Academy of Medical Sciences and Fellow of Oriel College, Oxford. He was recently appointed the founding Editor-in-Chief of the British Society for Immunology Journal *Immunotherapy Advances*.

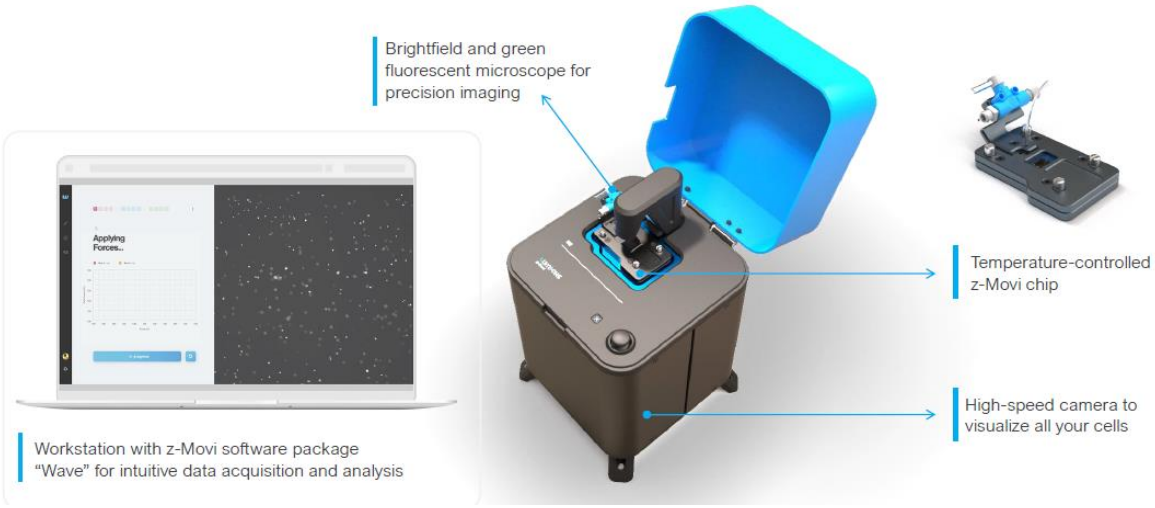
The Elliott lab takes an “atom-to-animal” approach to understanding how the presentation of antigens by MHC and MHC-like molecules influences the quality of T cell responses elicited

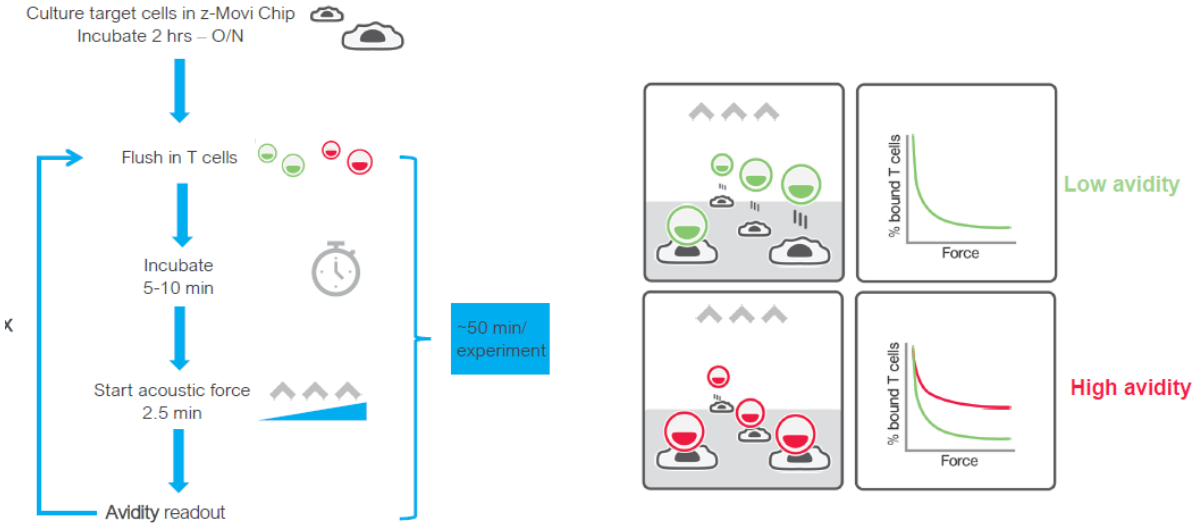
to cancer and infections. We collaborate widely so as to combine and focus diverse disciplines on this fundamental immunological process.

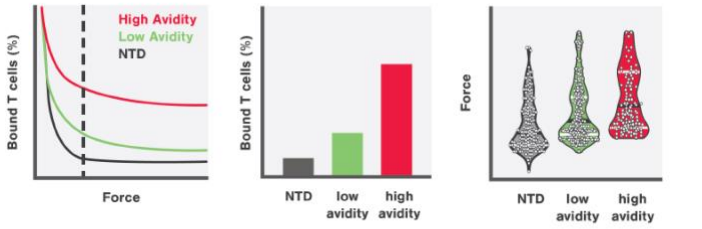
Publications relevant to this project

1. **Protective low avidity anti-tumour CD8+ T cells are selectively attenuated by regulatory T cells.** Sugiyarto G., Prosser D., Dadas O., Elliott T, James E. bioRxiv 481515; doi: https://doi.org/10.1101/481515 (submitted)
2. **HPV Epitope Processing Differences Correlate with ERAP1 Allotype and Extent of CD8+ T-Cell Tumor Infiltration in OPSCC** Reeves E, Wood O, Ottensmeier CH, King EV, Thomas GJ, Elliott T, James E. (2019) Cancer Immunol Res DOI: 10.1158/2326-6066.CIR-18-0498
3. **Induction of protective anti-tumor immunity through attenuation of ERAAP function.** James E., Bailey I., Sugiyarto G. and Elliott T.J. (2013) J Immunol. Jun 1;190(11):5839-46. doi: 10.4049/jimmunol.1300220
4. **CD8+ T-cell cross-competition is governed by peptide-MHC class I stability.** Galea I., Stasakova J., Dunscombe M.S., Ottensmeier C.H., Elliott T.J., Thirdborough S.M. (2012) *Eur J Immunol* 42(1):256
5. **Differential suppression of tumour specific CD8+ T cells by Regulatory T cells.** James E., Yeh A., King C., Korangy F., Bailey I., Murray N., Van den Eynde B. and Elliott T.J. (2010) J.Immunol. Nov 1;185(9):5048-55.

Appendix 1: Lumicks z-Movi platform







Avidity curves
Percentage of bound T cells as a function of force (NTD – negative control)

Bar graphs
Percentage effector cells that still bind after minimal force applied to release all unbound NTD effector cells

Violin dot plots
Force required to detach effector cells at single cell resolution

Project 16-2

Project Title: Factors that control T cell specificity in successful checkpoint blockade therapy

Supervisor: Prof Tim Elliott - T.J.Elliott@soton.ac.uk

Collaborators
Prof Mark Middleton (NDO) – Medical Oncology, experimental clinical trials
Prof Xin Lu (Ludwig Institute) – Cancer biology, deep phenotyping
Prof Tao Dong (NDM) – Cancer Immunology, Cytotoxic T cell function, epitope detection
Dr Nicola Ternette (NDM) – Immunopeptidomics and informatics
Prof. Mark Coles (Kennedy Institute) – Computational modelling

Background
A recent Phase 1/2 study (LUD2015-005; https://clinicaltrials.gov/ct2/show/NCT02735239) to evaluate the safety of durvalumab (anti-PD-L1) in combination with oxaliplatin/capecitabine chemotherapy in metastatic/locally advanced oesophageal cancer (OC) before surgery in operable OC, has provided an opportunity to survey the presentation of T cell epitopes (by immunopeptidomics) and analyse the immunophenotype of tumours (by RNAseq) prior to, during and after a 4-week therapeutic window of immunotherapy, prior to standard therapy. Of the nine patients retained on the study, 3 showed a partial clinical response, 3 had stable disease and disease progressed in two. Initial analysis has revealed that the response to immunotherapy correlates with HLA, with 4/6 patients showing some signs of response (PR or SD) expressing HLA-B*4402. Interestingly, the majority of HLA-B*4402 were also HLA-A*0201.

Extensive mechanistic studies in our lab have shown that HLA-B*4402 is highly dependent on the intracellular antigen processing and presentation cofactor tapasin. Tapasin works by catalysing protein dynamic changes that are associated with rapid intracellular peptide exchange, with the net effect of “filtering” HLA peptide cargo in favour of high affinity peptide ligands (epitopes). Other HLA alleles are less dependent on tapasin because, they are able to undergo the required protein dynamic changes spontaneously, though to a lesser extent compared to tapasin dependent alleles like HLA-B*4402. Consequently, these alleles experience less peptide filtering.

In a systems modelling approach, we have shown that the level of presentation of a given epitope is not only dependent on its intracellular availability and its affinity for HLA, but also on the extent to which its presenting HLA engages with tapasin-catalysed filtering. This factor can now be included in algorithms designed to predict likely T cell epitopes in cancer. The physiological significance of maintaining a spectrum of tapasin dependencies among alleles in a population is illustrated by a recent study in which we have shown that tapasin independence of HLA class I alleles correlates with a broader peptide repertoire and better control of HIV infection. Whether a similar correlation exists in T cell responses to cancer following immunotherapy is not known, though Chowell et al have reported a protective effect of the HLA-B44 supertype when the TCGA database was mined for response to checkpoint blockade therapy.

Proposal

The aim of this project is to determine the extent to which HLA tapasin dependence (and their combination with specific other HLA alleles) influences the size and shape of the cancer immunopeptidome, and whether these factors correlate with outcome following immunotherapy.

The key objectives are to:

1. Test the hypothesis, arising from computational modelling, that tapasin dependent HLA present a more "filtered" peptide repertoire: leading to a less diverse repertoire with more peptides reaching high abundance; whereas the repertoire of tapasin independent HLA is broader, with fewer peptides reaching high abundance.

2. Apply the peptide filter relation to immunopeptidomes generated by Dr Ternette from LUD2015-005 samples to identify the most abundantly presented tumour-specific peptides, and in collaboration with Prof Dong evaluate this as a method for predicting T cell responses in PBL from the same patients, and if possible determine the breadth of the anti-tumour response as a function of HLA tapasin dependence.

3. Mine available checkpoint blockade therapy databases, including TCGA (NGS, RNAseq of tumour samples) in order to test the hypothesis that the intensity of CD8+ T cell response (measured using key gene signatures) correlates with HLA tapasin dependency.

4. Mine these databases for evidence that HLA-A0201/HLA-B4402 individuals are enriched in the "successful CBT" group compared to HLA-A0201/non-B4402 and non-HLA-A0201/HLA-B4402. The reason for this is that in transplantation biology, transplanting an HLA-A0201+ organ into a B4402+ recipient increases the likelihood of rejection. The precise mechanism is not known, but it is thought that a T cell repertoire educated on HLA- B4402 contains a high proportion of TcR that cross react with HLA- A0201. So, in an individual who is HLA-A0201/B4402+ the hypothesis is that this high level of cross-reactivity will result in a high proportion of TcR with principal specificity for HLA-B4402-restricted peptide that are "borderline crossreactive" with HLA- A0201-restricted peptide. For this reason HLA-A*0201 restricted peptides will be screened in aim2.

Training Opportunities

This project is suitable for students who are interested in combining quantitative biological approaches to investigating human immune responses to cancer. A variety of methodologies will be used including informatics and computational modelling – involving both simulation and machine-learning. For this reason, this project is most suited to candidates with a strong background in computational approaches. Students will be registered on a techniques course in the first year which covers the latest techniques in cellular and molecular analysis, and training in the very latest experimental platforms such as immunopeptidomics is available via our collaborators in NDM. Taught modules in immunology are available in the university, and you will benefit from modular courses established for the Genomic Medicine and Statistics DPhil programme including bioinformatics and statistical genetics as well as functional genomics, at the Wellcome Centre for Human Genetics. You will also be trained in the

handling, processing and governance of human clinical samples and the principles of experimental medicine. Students will attend and participate in relevant seminar programmes within the NDM, WIMM , Ludwig Institute and NDO which provide the opportunity to meet and learn from international leaders in your field and build an exceptional academic network of contacts. Other specialist courses are available in Oxford and at other institutions in the UK, as necessary.

Supervisor Profile

Tim Elliott was amongst the key group of immunologists who developed the field of antigen presentation at the molecular level during the 1980s, undertaking a series of studies to determine and define the immunostimulatory properties of MHC Class I molecules and elucidating the molecular mechanisms of co-factor assisted peptide loading of MHC Class I in antigen presenting cells: work considered to be the fundamental foundation of much of the recent work on antigen presentation. The work underpins rational T-cell based vaccine design and continues to fuel translational research where discoveries in the areas of antigen discovery, T cell regulation and immunodominance are making a significant impact on new and ongoing cancer immunotherapy trials. His mechanistic studies have always benefitted from an active interface with the physical sciences including collaborations with synthetic and computational chemistry, computer science, mathematics and engineering. He is the founding Kidani Chair of Immune-oncology, a Fellow of the Royal Society for Biology and the Academy of Medical Sciences and Fellow of Oriel College, Oxford. He was recently appointed the founding Editor-in-Chief of the British Society for Immunology Journal *Immunotherapy Advances*.

The Elliott lab takes an "atom-to-animal" approach to understanding how the presentation of antigens by MHC and MHC-like molecules influences the quality of T cell responses elicited to cancer and infections. We collaborate widely so as to combine and focus diverse disciplines on this fundamental immunological process.

Publications relevant to this project

1. **HLA tapasin independence: broader peptide repertoire and HIV control.** Bashirova et al (2020) *Proc.Natl.Acad.Sci.USA* (in press)
2. **The role of MHC I protein dynamics in cofactor-assisted immunopeptidome editing**. Andy van Hateren and Tim Elliott (2020) *Current Opinions in Immunology* (in press)
3. **Protein Plasticity and Peptide Editing in the MHC I Antigen Processing Pathway.** Elliott, T. & Van Hateren, A. (2018). *Biochemistry*. 10: 1021-1023
4. **A mechanistic model for predicting cell surface presentation of competing peptides by MHC class I**. Denise Boulanger, Ruth C. Eccleston, Andrew Phillips, Peter V. Coveney, Neil Dalchau and Tim Elliott. (2018). *Front.Immunol.* 10.3389 doi.org/10.3389/fimmu.2018.01538
5. **Selector function of MHC I molecules is determined by protein plasticity.** Bailey A, Dalchau N, Carter R, Emmott S, Phillips A, Werner JM, Elliott T. (2015). *Sci. Rep.* 5, (14928) 1-15; doi: 10.1038/srep14928 (2015).

Project 17-1

Project Title: Analyses of paired host-virus genomic data to understand disease heterogeneity of viral infections.

Supervisor: Dr. Azim Ansari - azim.ansari@ndm.ox.ac.uk

Project Overview

Genome-wide association studies (GWAS) aim to identify the genetic basis of phenotypic traits using the variation that exists within natural populations. Uniquely for infectious diseases, the inter-individual heterogeneity in disease phenotype is linked to both host and pathogen genetic variation. Traditionally, genetic studies of infectious diseases have sought to explain between-individual variation in disease phenotypes by assessing genetic factors separately in humans or pathogens, under the assumption that these factors are independent. Although reasonable for some variants, there is strong theoretical and empirical evidence that genetic interactions between host and viruses play a major role in viral disease aetiology.

In this project you will integrate host and viral genomic data from the same patients to better understand viral pathogenesis and between-individual heterogeneity in disease outcomes. By analysis of paired host-virus genomic data from well-characterised cohorts you will gain novel insights on (a) host polymorphisms linked with viral sequence variation, (b) virus sites under strong host genetic selective pressures, (c) host and virus genetic factors independently contributing to disease phenotypes and (d) host-virus genetic interactions contributing to disease phenotypes. The findings have the potential to: (I) revolutionize our understanding of host-virus interactions and human biology; (II) aid in development of more effective vaccines, drug targets and immunotherapies; and (III) permit better use of therapies through patient stratification. In the age of “Big Data” and “Personalised Medicine”, analysis of paired host-pathogen genomic data will become increasingly important to uncover the mechanisms driving pathogen adaptations and heterogeneity of infection outcomes.

We have generated paired host-virus genomic data on a large cohort of HCV infected patients where associated clinical meta-data are available and are generating paired host-virus genomic data from well characterised cohorts infected with HBV and HIV and SARS-CoV-2. You will use these datasets to:

Aim 1. Define the host genomic pressures driving virus evolution across different host populations and virus lineages.

Aim 2. Identify host and virus genetic variants and interactions between the two that drive disease phenotypes.

Aim 3. Determine the host genetic variants and pathways linked to disease phenotypes across different viral infections.

Training opportunities
The student will develop expertise in Statistical genomics, Statistical Modelling, Machine Learning, bioinformatics, infectious diseases, evolution and population genetics. This studentship will be based at the Peter Medawar Building for Pathogen Research (PMB) and Big Data Institute (BDI) at Oxford. The PMB houses around 150 scientists working on HIV, HCV, influenza, TB, malaria, SARS-CoV-2 and dengue and many of the PIs are global leaders in the study of infections. BDI focuses on the analysis of large and complex datasets with an emphasis on infectious diseases. Academic excellence at the PMB and BDI is reflected in recent major papers in Nature, Science, Cell, Nature Genetics and other high profile journals.

Supervisor
Dr. Azim Ansari has a DPhil in Statistical Genetics from University of Oxford and his groups focus is understanding infectious diseases with bringing together heterogenous data types. He has published one of the first host-to-virus genome-to-genome association studies (Ansari et al. Nature Genetics 2017) and has shown that host innate immune system has a large impact on HCV sequence diversity (Ansari et al. eLife 2019). Other research work includes identification of novel polymorphisms in HCV that significantly reduce success rate of sofosbuvir treatment (Smith et al. bioRxiv 2020) and development of statistical methods and software which detect lineages on a phylogenetic tree that are associated with a phenotype (Ansari et al. Genetics 2016; Behr et al. PNAS 2020). You will be working in a highly productive and inter-disciplinary research environment focusing on host-pathogen interaction studies.

Co-supervisors:
Prof. Eleanor Barnes has a long-standing interest in HBV/HCV viral pathogenesis, immunology and vaccine development, and personalised medicine. She leads a research group with a focus on T cell immunity and viral control, in association with viral genomic analysis. She has led the laboratory work into human experimental medicine studies with the aims of developing an HBV simian adenoviral vectored vaccines for HBV immunotherapy. She has published >140 primary research peer-reviewed journal articles (with additional chapters and reviews) primarily in the field of hepatitis, vaccinology and hepatology more broadly. Her research is consistently published in the leading specialist journals, most recently in Science Translational Medicine, Hepatology, and Nature Genetics (all as lead and/or corresponding author).

Prof. Philip Goulder has used HIV as a model to understand the impact of pediatric versus adult immunity, and of immune sex differences from conception onwards, on outcome of infectious disease. Although immune sex differences are often considered to have impact only in adulthood, they are evident throughout childhood and have very large impact on outcome from the range of childhood infections (review: Muenchhoff & Goulder, J Infect Dis, 2014). More recently we have shown that immune sex differences arise in utero and are important: the female fetus is 2-3 times more susceptible to HIV infection in utero if the mother herself becomes infected during pregnancy and females are also twice as susceptible to mother-to-child transmission of HCV. The mechanism is related to the selective transmission of interferon type I-resistant mutants specifically to female fetuses (Adland et al, Nat Comm, 2020).

Key publications

1. Simmonds P, Kuypers L, Irving WL, McLauchlan J, Cooke GS, Barnes E, **Ansari MA**. Impact of virus subtype and host IFNL4 genotype on large-scale RNA structure formation in the genome of hepatitis C virus. RNA. 2020 Aug 3:rna-075465.

2. Behr M, **Ansari MA**, Munk A, Holmes C. Testing for dependence on tree structures. Proceedings of the National Academy of Sciences. 2020 May 5;117(18):9787-92.

3. **Ansari MA**, Aranday-Cortes E, Ip C, da Silva Filipe A, Hin L, Bamford C, Bonsall D, Trebes A, Piazza P, Sreenu V, Cowton V, STOP-HCV Consortium, Hudson E, Bowden R, Patel A, Foster G, Irving W, Agarwal K, Thomson E, Simmonds P, Klenerman P, Holmes C, Barnes E, Spencer C, McLauchlan J, Pedergnana V. Interferon lambda 4 impacts on the genetic diversity of hepatitis C virus. Elife, 2019 Sep; 8. pii: e42463. doi: 10.7554/eLife.42463.

4. **Ansari MA*,** Pedergnana V*, L C Ip C, Magri A, Von Delft A, Bonsall D, Chaturvedi N, Bartha I, Smith D, Nicholson G, McVean G, Trebes A, Piazza P, Fellay J, Cooke G, Foster GR; STOP-HCV Consortium, Hudson E, McLauchlan J, Simmonds P, Bowden R, Klenerman P, Barnes E, Spencer CCA. Genome-to-genome analysis highlights the effect of the human innate and adaptive immune systems on the hepatitis C virus. Nature Genetics. 2017 May;49(5):666-673. doi: 10.1038/ng.3835.

5. **Ansari MA**, Didelot X. Bayesian Inference of the Evolution of a Phenotype Distribution on a Phylogenetic Tree. Genetics. 2016 Sep;204(1):89-98. doi: 10.1534/genetics.116.190496.

Project 17-2

Project Title: Understanding mechanisms of sex disparities in infectious diseases

Supervisor: Dr. Azim Ansari - azim.ansari@ndm.ox.ac.uk

Project Overview

The mortality rate for COVID-19 pandemic has been two-fold higher in men than women. Similar observation extends to susceptibility and outcome of most other infectious diseases. For instance, after initial Hepatitis C Virus infection women are 2-3 times more likely to spontaneously clear the virus without any interventions and in HIV infection females are 5 times more likely to achieve elite control (complete suppression virus without therapy) than men. However, a consequence of the more vigorous immune response observed in females is more immunopathology and auto-immune diseases (such as lupus) in women than men. For the same reasons, females make stronger immune responses to vaccines but suffer more adverse events. Despite large evidence for sex differences in autoimmune diseases and susceptibility and outcome of infectious diseases, data addressing the biological mechanism are remarkably scarce.

In this project you will use computational and experimental methods to probe differences in immune system that lead to sex differences in infectious diseases. We will investigate this question across many infections including HCV, HBV, HIV and COVID-19. You will start with analysing the available RNA-seq and genomic data from our cohorts and other public databases to understand the role of heterogeneity in X chromosome inactivation in female immune cells and the transcriptional consequence and its contribution to better outcome in infectious diseases. In the next stage you will stimulate male and female immune cells with different immunogens and perform single cell RNA-sequencing to evaluate differential responses across distinct cell types and their association with sex. The project will also use samples and data from vaccine clinical trials. The baseline samples will be compared to the post-vaccination samples and differences in immune systems between sexes will be investigated.

Training opportunities

The student will develop expertise in Statistical genomics, Statistical Modelling, Machine Learning, bioinformatics, infectious diseases, evolution and population genetics. This studentship will be based at the Peter Medawar Building for Pathogen Research (PMB) and Big Data Institute (BDI) at Oxford. The PMB houses around 150 scientists working on HIV, HCV, influenza, TB, malaria, SARS-CoV-2 and dengue and many of the PIs are global leaders in the study of infections. BDI focuses on the analysis of large and complex datasets with an emphasis on infectious diseases. Academic excellence at the PMB and BDI is reflected in recent major papers in Nature, Science, Cell, Nature Genetics and other high profile journals.

Supervisor

Dr. Azim Ansari has a DPhil in Statistical Genetics from University of Oxford and his groups focus is understanding infectious diseases with bringing together heterogenous data types. He has published one of the first host-to-virus genome-to-genome association studies (Ansari et al. Nature Genetics 2017) and has shown that host innate immune system has a large

impact on HCV sequence diversity (Ansari et al. eLife 2019). Other research work includes identification of novel polymorphisms in HCV that significantly reduce success rate of sofosbuvir treatment (Smith et al. bioRxiv 2020) and development of statistical methods and software which detect lineages on a phylogenetic tree that are associated with a phenotype (Ansari et al. Genetics 2016; Behr et al. PNAS 2020). You will be working in a highly productive and inter-disciplinary research environment focusing on host-pathogen interaction studies.

Co-supervisors:
Prof. Philip Goulder has used HIV as a model to understand the impact of pediatric versus adult immunity, and of immune sex differences from conception onwards, on outcome of infectious disease. Although immune sex differences are often considered to have impact only in adulthood, they are evident throughout childhood and have very large impact on outcome from the range of childhood infections (review: Muenchhoff & Goulder, J Infect Dis, 2014). More recently we have shown that immune sex differences arise in utero and are important: the female fetus is 2-3 times more susceptible to HIV infection in utero if the mother herself becomes infected during pregnancy and females are also twice as susceptible to mother-to-child transmission of HCV. The mechanism is related to the selective transmission of interferon type I-resistant mutants specifically to female fetuses (Adland et al, Nat Comm, 2020).

Prof. Eleanor Barnes has a long-standing interest in HBV/HCV viral pathogenesis, immunology and vaccine development, and personalised medicine. She leads a research group with a focus on T cell immunity and viral control, in association with viral genomic analysis. She has led the laboratory work into human experimental medicine studies with the aims of developing an HBV simian adenoviral vectored vaccines for HBV immunotherapy. She has published >140 primary research peer-reviewed journal articles (with additional chapters and reviews) primarily in the field of hepatitis, vaccinology and hepatology more broadly. Her research is consistently published in the leading specialist journals, most recently in Science Translational Medicine, Hepatology, and Nature Genetics (all as lead and/or corresponding author).

Key publications

1. Simmonds P, Kuypers L, Irving WL, McLauchlan J, Cooke GS, Barnes E, **Ansari MA**. Impact of virus subtype and host IFNL4 genotype on large-scale RNA structure formation in the genome of hepatitis C virus. RNA. 2020 Aug 3:rna-075465.

2. Behr M, **Ansari MA**, Munk A, Holmes C. Testing for dependence on tree structures. Proceedings of the National Academy of Sciences. 2020 May 5;117(18):9787-92.

3. **Ansari MA**, Aranday-Cortes E, Ip C, da Silva Filipe A, Hin L, Bamford C, Bonsall D, Trebes A, Piazza P, Sreenu V, Cowton V, STOP-HCV Consortium, Hudson E, Bowden R, Patel A, Foster G, Irving W, Agarwal K, Thomson E, Simmonds P, Klenerman P, Holmes C, Barnes E, Spencer C, McLauchlan J, Pedergnana V. Interferon lambda 4 impacts on the genetic diversity of hepatitis C virus. Elife, 2019 Sep; 8. pii: e42463. doi: 10.7554/eLife.42463.

4. **Ansari MA*,** Pedergnana V*, L C Ip C, Magri A, Von Delft A, Bonsall D, Chaturvedi N, Bartha I, Smith D, Nicholson G, McVean G, Trebes A, Piazza P, Fellay J, Cooke G, Foster GR; STOP-HCV Consortium, Hudson E, McLauchlan J, Simmonds P, Bowden R, Klenerman P, Barnes E, Spencer CCA. Genome-to-genome analysis highlights the effect of the human innate and adaptive immune systems on the hepatitis C virus. Nature Genetics. 2017 May;49(5):666-673. doi: 10.1038/ng.3835.

5. **Ansari MA**, Didelot X. Bayesian Inference of the Evolution of a Phenotype Distribution on a Phylogenetic Tree. Genetics. 2016 Sep;204(1):89-98. doi: 10.1534/genetics.116.190496.

6. Vieira VA, Zuidewind P, Muenchhoff M, Roider J, Millar J, Clapson M, Van Zyl A, Shingadia D, Adland E, Athavale R, Grayson N, **Ansari MA**, et al. Strong sex bias in elite control of paediatric HIV infection. AIDS (London, England). 2019 Jan 2;33(1):67.

7. Adland E, Millar J, Bengu N, Muenchhoff M, Fillis R, Sprenger K, Ntlantsana V, Roider J, Vieira V, Govender K, Adamson J, Nxele N, Ochsenbauer C, Kappes J, Mori L, van Lobenstein J, Graza Y, Chinniah K, Kapongo C, Bhoola R, Krishna M, Matthews PC, Poderos RP, Lluch MC, Puertas MC, Prado JG, McKerrow N, Archary M, Ndung'u T, Groll A, Jooste P, Martinez-Picado J, Altfeld M, **Goulder PJR.** Sex-specific innate immune selection of HIV-1 in utero is associated with increased female susceptibility to infection. ***Nat Commun***. 2020 May 4;11(1):2257. doi: 10.1038/s41467-020-16215-7.

8. Muenchhoff M, **Goulder PJR**. Sex differences in pediatric infectious diseases. ***J Infect Dis***. 2014 Jul 15;209 Suppl 3:S120-6.

Project 18

Project Title: Development of CRISPR/Cas-based gene editing therapies for age-related macular degeneration

Supervisor: Dr Kanmin Xue - kanmin.xue@eye.ox.ac.uk

Project Overview

The retina provides the ideal organ for testing novel genetic therapies. Clinical trials of adeno-associated viral (AAV) vector-mediated gene augmentation therapies have demonstrated efficacy and safety in the treatment of monogenic inherited retinal diseases, including Leber congenital amaurosis, choroideremia and X-linked retinitis pigmentosa. However, the disease targets for gene augmentation are limited by the coding capacity of AAV (~4.7kb). By combining the AAV gene delivery platform with the latest CRISPR/Cas gene editing systems and synthetic oligonucleotide design, we aim to develop novel disease-modifying therapies for a broad range of retinal diseases, in particular, age-related macular degeneration (AMD).

AMD is one of the leading causes of blindness, affecting 2.1% of the population over the age of 50, rising to 12% in those over the age of 80. It is characterised by drusen deposition within the retina, and degeneration of the retinal pigment epithelium (RPE) and photoreceptors. The pathogenesis is multifactorial with several key risk factors: (i) age; (ii) oxidative stress (e.g. smoking); and (iii) genetic polymorphisms in complement factors (H, B & C2), serine protease HTRA1, apolipoprotein E (ApoE), and age-related maculopathy susceptibility protein 2 (ARMS2). There is currently no treatment for the majority (95%) of AMD which are of the atrophic (dry) type, while the management of exudative (wet) AMD (5% of cases) relies on long-term intravitreal injections of anti-vascular endothelial growth factors (anti-VEGFs).

This project will focus on the following three main objectives.

1. Use mouse models to study the link between genetic polymorphisms of the ApoE allele and amyloid-β deposition in the pathogenesis of AMD.

Amyloid-β is an important constituent of drusen, the pathological hallmark of AMD, yet its role in AMD pathogenesis is unclear. The ApoE4 allele is known to confer high risk for Alzheimer's disease but low risk for AMD. In contrast, the ApoE2 allele carries high risk for advanced AMD but low risk for Alzheimer's. Understanding this genetic paradox will improve our understanding of the pathogenesis of both types of neurodegenerations.

2. Design and test novel modes of delivering CRISPR/Cas gene editing systems to target key pathogenic factors in AMD.

The design of CRISPR/Cas-based gene knockout or base editing systems will require a number of considerations, including guide RNA design, selection of Cas9 orthologue/effector and promoter. The gene editing system may be designed for delivery via viral vector, nanoparticles or hybrid methods involving synthetic oligonucleotides. These gene editing systems will be tested in cell lines and using reporter assays to provide 'proof-of-concept'.

3. Testing of AMD disease-modifying gene editing therapy *ex vivo* and *in vivo* to provide pre-clinical data to support future clinical trials.

CRISPR/Cas based gene editing therapies will be tested in human tissue/organoids and non-human primate models to assess their on-target editing efficiency (clinical viability) and off-target effects (safety), thus providing key pre-clinical data to facilitate human trials.

Training opportunities

The student will receive training in state-of-the-art CRISPR/Cas gene editing technologies, AAV vector design/production, synthetic oligonucleotides, molecular biology and animal experimental techniques relevant to vision research. The student will also be encouraged to obtain general training in statistical analysis, bioinformatics, machine learning, and other aspects of clinician scientist career development through the University of Oxford. In addition, the student will have opportunities to collaborate with other members of the group on relevant scientific and clinical projects, including those of Professor Robert MacLaren's team (with whom we have close collaborations), the clinical ophthalmology and clinical trials teams based at the Oxford Eye Hospital (all within the same building). We expect the student to prepare papers for publication in high impact journals and present regularly at international conferences.

Supervisor/short profile

Kanmin Xue MA(*Oxon*) MB BChir PhD (*Cantab*) FRCOphth is a Wellcome Trust clinician scientist fellow at the University of Oxford and Honorary Consultant Vitreoretinal Surgeon at the Oxford Eye Hospital. He leads the **Retinal Disease and Repair Group** in the Nuffield Laboratory of Ophthalmology. His lab is currently focused on investigating the mechanisms of retinal inflammation (uveitis) and degeneration (age-related macular degeneration), and developing novel gene therapies for these retinal diseases. He previously held the role of National Institute for Health Research (NIHR) Academic Clinical Lecturer in Oxford between 2014 and 2018, and led the world's first retinal gene therapy clinical trials for choroideremia and X-linked retinitis pigmentosa and first-in-human trial of robot-assisted retinal surgery under the mentorship of Professor Robert MacLaren FMedSci. He has completed the UK specialist training in ophthalmology and a vitreoretinal fellowship at the Royal Victorian Eye and Ear Hospital in Melbourne, Australia. He previously conducted PhD research with Professor Michael Neuberger FRS at the **MRC Laboratory of Molecular Biology** in Cambridge into how DNA editing by activation-induced deaminase brings about antibody class switching, underwent clinical training at Trinity College Cambridge and London, and pre-clinical training at Brasenose College Oxford where he was awarded Martin Wronker Prize for the top first class in Medicine.

Key scientific achievements

- The first phase I/II clinical trial of retinal gene therapy for X-linked retinitis pigmentosa associated with mutations in *RPGR* (one of the most common forms of retinitis pigmentosa), showing significant improvement in retinal sensitivity, visual field and retinal anatomy.
- The first complete phase I/II clinical trial of retinal gene therapy for choroideremia, meeting the primary endpoint of significant improvement in visual acuity which is sustained up to 5 years.

- The first-in-human trial of robot-assisted retinal surgery, demonstrating safety and clinical potential.
- Demonstration of retinal innate immunity to adeno-associated virus (AAV) as an important limiting factor for viral transduction, leading to discovery of hydroxychloroquine as an adjunct for improving the clinical efficacy of gene therapy.
- Demonstration that antibody class switching occurs through cytokine-directed activation-induced deaminase (AID)-mediated mutations within switch regions of immunoglobulin heavy chain locus.

Awards and honours

- Wellcome Trust Clinical Research Career Development Fellowship – Stage 2 (2019)
- Ruskell Medal, Worshipful Company of Spectacle Makers, City of London (2019)
- Ian Fraser Cup, the 101st Oxford Ophthalmological Congress, UK (2017)
- Luigi Barca Award, Florence Retina Meeting (FLOREtina), Italy (2017).
- University of Oxford Martin Wronker Prize in Medicine (top first class in the year) (2003)

Clinical supervisors

The PI is a clinician scientist. Opportunity for co-supervision available within the Nuffield Laboratory of Ophthalmology and University of Oxford, in particular, with key collaborator, Professor Robert MacLaren.

Key publications (up to 5)

1. Quinn J & Musa A, Kantor A, McClements M, Cehajic-Kapetanovic J, MacLaren RE, **Xue K**. Genome editing strategies for treating human retinal degenerations. ***Human Gene Therapy***. 2020. Doi: 10.1089/hum.2020.231. An overview of CRISPR-Cas based gene editing approaches for treating retinal diseases that we are developing in the lab.

2. Cehajic-Kapetanovic J & **Xue K** (joint first authors), Martinez-Fernandez de la Camara C, Nanda A, Davies A, Wood L, Salvetti AP, Fischer MD, Aylward JW, Barnard AR, Jolly JK, Luo E, Lujan BJ, Ong T, Girach A, Black, Gregory NZ, Davies JL, Rosa PR, Lotery AJ, Lam BL, Stanga PE, MacLaren RE. Initial results from a first-in-human gene therapy trial on X-linked retinitis pigmentosa caused by mutations in RPGR. ***Nature Medicine***. 2020;26:354–359.

3. Chandler LC, Barnard AR, Caddy SL, Patrício MI, Fu H, Rada C, MacLaren RE, **Xue K**. Enhancement of adeno-associated viral (AAV) gene therapy using hydroxychloroquine in murine and human tissues. ***Molecular Therapy – Methods Clin Dev***. 2019;14:77-89. Demonstration of retinal innate immunity to adeno-associated virus (AAV) as an important limiting factor for viral transduction, leading to discovery of hydroxychloroquine as an adjunct for improving the clinical efficacy of gene therapy.

4. **Xue K**, Jolly JK, Barnard AR, Rudenko A, Salvetti AP, Patrício MI, Edwards TL, Groppe M, Tolmachova T, Black GC, Webster AR, Lotery AJ, Holder GE, Downes SM, Seabra MC, MacLaren RE. Beneficial effects on vision in patients undergoing retinal gene therapy for choroideremia. ***Nature Medicine***. 2018;24:1507-1512. Complete results

of the world's first phase I/II clinical trial of retinal gene therapy for choroideremia, an important inherited cause of blindness. By demonstrating sustained preservation or improvement in visual acuity in the treated eyes over 2 to 5 years, the results led to the largest international multi-centre phase III trial of gene therapy to date, and helped to establish the protocols for evaluating changes in visual function and retinal anatomy in future retinal gene therapy trials.

5. Edwards TL, **Xue K**, Meenink TC, Beelen MJ, Naus G, Simunovic MP, Latasiewicz M, Farmery AD, de Smet MD, MacLaren RE. First-in-human study of the safety and viability of intraocular robotic surgery. ***Nature Biomedical Engineering***. 2018;2:649-656. The world's first clinical trial of vitreoretinal surgery using a telemanipulated robot, demonstrating safety and clinical potential.