**Project 1**

**Project Title:** Understanding the cellular heterogenoty of vaccine induced T cell responses in the liver in order to develop new Immunotherapeutic strategies against hepatitis B virus infection

**Supervisor:** Professor Ellie Barnes - [ellie.barnes@ndm.ox.ac.uk](mailto:ellie.barnes@ndm.ox.ac.uk)

**Project Overview**

Deaths from liver disease caused by viral hepatitis and hepatocellular cancer have reached epidemic proportions globally. Viral hepatitis alone causes 1.2 million deaths each year. In recent years, my group has led an internationally pioneering program, developing viral vectored T cell vaccines to tackle viral hepatitis in pre-clinical rodent models and human studies. Using these technologies, we are now able to generate unprecedented levels of antigen specific T cell immune responses in naïve animals and healthy humans. However, applying the same technologies in chronic liver disease and cancer, has revealed the enormous challenges in restoring immunity in the context of ongoing antigen exposure and the immune regulatory liver microenvironment. This DPhil aims to unravel the fate of vaccine induced T cells as they meet the liver immunoregulatory environment.

Building on our experience in viral vectored vaccine technology, the immunobiology of viral hepatitis and liver disease, you will define the cellular heterogeneity of viral antigen specific T cell populations that are primed in the periphery and determine the fate and function of these as they migrate into healthy and chronically infected liver. For this you will use potent T cell vaccines in 1) rodent models and 2) both healthy and HBV infected humans (using sampled from a phase I human study that will begin in 2019 extending through 2020).

This analysis will be done at extraordinary resolution, using unbiased single cell RNA sequencing (sc-RNAseq) combined with MHC class I/II multimer technology with phenotypic and functional T cell assays. A comparative analysis of differentially expressed (DE) genes will be identified in clusters (using t-SNE), with mapping of vaccine induced T cell populations, in blood, naïve and virally infected livers. Genes and/or markers from cell-specific populations using publically available gene data sets or flow cytometry will be used to further define cell populations. We will determine the transcription factors that allow vaccine induced T cells to maintain effector function and tissue residence (TRM) in the regulatory immune liver environment (or not). Liver TRM have recently been shown to express distinct adhesion molecules (e.g. LFA-1) that will provide land mark gene in our analysis. Exactly where and how TRM are generated in vivo after vaccination, and how they function in response to viral challenge will be a focus of the DPhil. The effects of check point modulators on vaccine induced T cell responses may also be explored.

**Training opportunities**

The student’s PhD will give him/her a superb training in liver immunology and vaccinology, including advanced single cell (sc)-RNA sequencing genomic analysis and bioinformatic pipelines combined with advanced conventional immune cell functional and phenotypic analysis in flow cytometric assays. This studentship will be based at the Peter Medawar Building (PMB) for Pathogen Research (PMB) equipped with state-of-the art facilities, Cat-3 laboratories, and dedicated laboratory space for vaccine development. The PMB houses around 150 scientists working on HIV, HCV, influenza, TB, malaria and dengue.  There is also a powerful section devoted to statistical genetics closely integrated into the science of pathogens.  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.

**Supervisor**

Prof. Eleanor Barnes has a 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 a 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. Kelly C, Swadling L, Capone S, Brown A, Richardson R, Halliday J, von Delft A, Oo Y, Mutimer D, Kurioka A, Hartnell F, Collier J, Ammendola V, Del Sorbo M, Grazioli F, Esposito ML, Di Marco S, Siani L, Traboni C, Hill AV, Colloca S, Nicosia A, Cortese R, Folgori A, Klenerman P, **Barnes E.** [Chronic hepatitis C viral infection subverts vaccine-induced T-cell immunity in humans.](https://www.ncbi.nlm.nih.gov/pubmed/26474390) ***Hepatology.*** 2016 May;63(5):1455-70.

*Detailed demonstration of the interplay between T cells generated by vaccination and circulating HCV virus-shows the need for a vaccine to target HCV quasispecies.*

1. [von Delft A](http://www.ncbi.nlm.nih.gov/pubmed/?term=von%20Delft%20A%5BAuthor%5D&cauthor=true&cauthor_uid=26092843), [Humphreys IS](http://www.ncbi.nlm.nih.gov/pubmed/?term=Humphreys%20IS%5BAuthor%5D&cauthor=true&cauthor_uid=26092843), [Brown A](http://www.ncbi.nlm.nih.gov/pubmed/?term=Brown%20A%5BAuthor%5D&cauthor=true&cauthor_uid=26092843), [Pfafferott K](http://www.ncbi.nlm.nih.gov/pubmed/?term=Pfafferott%20K%5BAuthor%5D&cauthor=true&cauthor_uid=26092843), [Lucas M](http://www.ncbi.nlm.nih.gov/pubmed/?term=Lucas%20M%5BAuthor%5D&cauthor=true&cauthor_uid=26092843), [Klenerman P](http://www.ncbi.nlm.nih.gov/pubmed/?term=Klenerman%20P%5BAuthor%5D&cauthor=true&cauthor_uid=26092843), [Lauer GM](http://www.ncbi.nlm.nih.gov/pubmed/?term=Lauer%20GM%5BAuthor%5D&cauthor=true&cauthor_uid=26092843), [Cox AL](http://www.ncbi.nlm.nih.gov/pubmed/?term=Cox%20AL%5BAuthor%5D&cauthor=true&cauthor_uid=26092843), [Gaudieri S](http://www.ncbi.nlm.nih.gov/pubmed/?term=Gaudieri%20S%5BAuthor%5D&cauthor=true&cauthor_uid=26092843), [**Barnes E**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Barnes%20E%5BAuthor%5D&cauthor=true&cauthor_uid=26092843). The broad assessment of HCV genotypes 1 and 3 antigenic targets reveals limited cross-reactivity with implications for vaccine design. ***Gut.*** 2016 Jan;65(1):112-23.

*This shows that there is minimal overlap in T cell immunity between circulating major genotypes in natural infection. A vaccine to a single genotype may not therefore afford protection.*

1. 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.](http://www.ncbi.nlm.nih.gov/pubmed/25378645) ***Sci Transl Med***. 2014 Nov 5;6(261)

*The key paper that shows detailed T cell function and phenotype in humans after heterologous Ad/MVA prime boost vaccination-this vaccine is now in phase-II efficacy testing.*

1. Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, **Barnes E**. [Global distribution and prevalence of hepatitis C virus genotypes.](http://www.ncbi.nlm.nih.gov/pubmed/25069599) ***Hepatology.*** 2014 Jul 28.

*The most highly cited paper of 2014-2015 in hepatology showing the global distribution of HCV genotypes-the driver for a pan-genotypic HCV vaccine*

**Project 2**

**Project Title:** Creating biochemical and cellular models of N-glycosylation diseases

**Supervisor:** Dr Yin Yao Dong - [yin.dong@ndcn.ox.ac.uk](mailto:yin.dong@ndcn.ox.ac.uk)

**Project Overview**

N-glycosylation is one of the most common forms of post-translational modification, that is essential to the function of all human cells. However, the roles of most N-glycans are unknown. Due to its importance, and ubiquitous presence, abnormalities in N-glycosylation can lead to a diverse array of clinical symptoms. Most patients with mutations in N-glycosylation genes suffer from the devastating multisystem disorders congenital diseases of glycosylation (CDG). Interestingly, others develop the milder congenital Myasthenic syndromes (CMS), where patients mainly suffer from abnormal neuromuscular junction (NMJ) development and function. Due to the diversity of symptoms, N-glycosylation disorders are still underdiagnosed in Europe and US, and much more so in China. Very few treatment options are available for most CDG and glycosylation-CMS patients, and almost all of these are symptomatic.

The aim of this project is to create biochemical and cellular models of N-glycosylation diseases, such as CMS and CDG caused by mutations in DPAGT1, ALG6, ALG14, GFPT1 and GMPPB. The biochemical models will allow us to better understand how disease causing mutations alter protein function, and the dynamics between enzymes of the N-glycosylation pathway. The cellular models will allow us to study how mutations affect the glycosylation of key NMJ proteins, thus determining the importance of N-glycosylation to the NMJ, and the pathogenic mechanisms of disease. These models will also be used as platforms for testing new therapeutic strategies.

**Training opportunities**

The candidate will have the unique opportunity to work with scientists from 4 leading laboratories, with a diverse range of expertise, as well as observe the work of expert clinicians at specialist CMS clinics. They will be based in the world renowned Weatherall Institute of Molecular Medicine, with access to all its state of the art core facilities such as super-resolution microscopy, flow cytometry, genome engineering, mass cytometry, single cell genomics etc (<https://www.imm.ox.ac.uk/research/facilities>). The candidate will learn to use cutting edge high throughput protein production methods to express and purify various different N-glycosylation enzymes to set up biochemical models of disease. Using biochemical and biophysical methods, they will assess how disease associated mutations affect protein function, interactions and stability. The candidate will learn to work with immortalised patient myocytes to create cell culture models of CMS. Where patient cells are unavailable, they will use CRISPR/CAS9 to introduce patient genotypes into mouse myocytes to re-create disease phenotypes. The candidate will learn a wide range of protein purification methods to purify glycoproteins from the cultured cells, for glycomics and glycoproteomics studies in collaboration with Dr Stuart Haslam of Imperial College.

The candidate will have opportunities to attend specialist CMS clinics to observe how leading experts diagnose and treat CMS patients, as well as attend the weekly grand round teaching sessions on neurological disorders. There will also be opportunities to work with Prof Hudson Freeze in the US, who is a world renowned expert in CDG research.

**Supervisor**

Dr Yin Yao Dong was recently awarded a prestigious MRC Career Development fellowship to study the importance of N-glycosylation in the NMJ, in collaboration with Prof David Beeson, Prof Liz Carpenter, Dr Stuart Haslam, and Prof Hudson Freeze. He previously conducted his post-doctoral research at the Structural Genomics Consortium, working on the structure and function of human membrane proteins. Dr Dong holds a Bsc 1st class from the University of Warwick, and undertook his PhD training at the University of Birmingham with Prof Karen Morrison.

**Clinical Supervisors**

Prof David Beeson and Dr Jackie Palace are renowned experts in CMS. Dr Palace runs weekly specialist CMS clinics at the John Radcliffe Hospital in Oxford, and Prof Beeson runs the CMS diagnostic laboratory. Together, they devised many of the modern treatments that have been life changing for hundreds of CMS patients.

**Key Publications**

1. **Dong YY\*,** Wang H\*, Pike AC\*, Cochrane SA, Hamedzadeh S, Wyszyński FJ, Bushell SR, Royer SF, Widdick DA, Sajid A, Boshoff HI, Park Y, Lucas R, Liu WM, Lee SS, Machida T, Minall L, Mehmood S, Belaya K, Liu WW, Chu A, Shrestha L, Mukhopadhyay SMM, Strain-Damerell C, Chalk R, Burgess-Brown NA, Bibb MJ, Barry Iii CE, Robinson CV, Beeson D, Davis BG, Carpenter EP. (2018) “Structures of DPAGT1 explain glycosylation disease mechanisms and advance TB antibiotic design” ***Cell***. 2018 Nov 1;175(4):1045-1058.e16
   * Joint 1st authors
2. **Dong YY**\*, Pike AC\*, Mackenzie A, McClenaghan C, Aryal P, Dong L, Quigley A, Grieben M, Goubin S, Mukhopadhyay S, Ruda GF, Clausen MV, Cao L, Brennan PE, Burgess-Brown NA, Sansom MS, Tucker SJ, Carpenter EP (2015) “K2P channel gating mechanisms revealed by structures of TREK-2 and a complex with Prozac” ***Science*** *347(6227):1256-9*
   * Joint 1st authors
3. Quigley A\*, **Dong YY**\*, Pike AC\*, Dong L, Shrestha L, Berridge G, Stansfeld PJ, Sansom MS, Edwards AM, Bountra C, von Delft F, Bullock AN, Burgess-Brown NA, Carpenter EP. (2013) “The structural basis of ZMPSTE24-dependent laminopathies” ***Science*** *339(6127):1604-7*
   * Joint 1st authors

**Project 3-1**

**Project Title:** The role of zinc in immune function

**Supervisor:** Professor Richard Cornall - [richard.cornall@ndm.ox.ac.uk](mailto:richard.cornall@ndm.ox.ac.uk)

**Project Overview**

Investigating human immunodeficiency is a good way to discover new immune functions and this year we described a novel form of inherited B cell deficiency caused by hypomorphic mutations in an intracellular zinc transporter, ZIP7. The study was led by Consuelo Anzilotti, a former postdoc in the lab, who used CRISPR to generate animal models of the human disease (***Anzilotti C et al, Nature Immunol 20:350-361, 2019***). Our findings suggest the defect in the positive selection of developing B cells is due to a defect in B cell receptor (BCR) signalling. This is associated with increased phosphatase activity, which is associated with reduced cytoplasmic zinc levels.

Our discovery opens an important and new area of investigation. Globally, 17% of the world’s population is thought to be zinc deficient, and zinc deficiency has long been associated with immune dysfunction; but the causes have always been unclear.

The aim of this graduate project is to study the role of zinc in immune function and disease, starting with further study of ZIP7. To make this possible, we will start with a series of mice with further ZIP7 mutant alleles, which also recapitulate the human disease but have different downstream effects. We have also created a B-cell specific inducible knockout of ZIP7, so we can explore its function in the B cell response to antigen and positive selection in the germinal centre.

Our proposal is that the first year will be spent characterising the animals and cell lines and generating more tools. Our laboratory is part of the MRC Human Immunology Unit (MRC HIU), in the Weatherall Institute of Molecular Medicine (WIMM) and the Wellcome Trust Centre for Human Genetics (WGH), where we are located. We are well supported with access to cutting edge reagents and technology. There will be several challenging and ground-breaking parts to the project, which will require the student to develop approaches to the measurement of rare elements, imaging using FRET-FLIM and the study of rare populations of cells. There will be strong support from the laboratory, supervisors and the wider community in the WIMM, WGH and in Oxford.

The student will study the affected cells at a molecular level, using biochemistry, proteomics and genetics to build and address hypotheses. We expect the study to provide new insight into immune mechanisms and influence the treatment of patients.  Our lab website is at **www.ccmp.ox.ac.uk/cornall-group**.

**Training Opportunities**

This programme is suitable for any student who is interested in using cellular, genetic and biochemical approaches to studying human disease. A wide variety of lab techniques will be used and you will be become expert in cell biology and immunology. Experience of immunology is not a requirement, since teaching is available in the University and lab. Through our membership of the WGH and the MRC HIU, we have access to training in single cell approaches, bioinformatics, high resolution imaging, the use of animal models and other techniques. Students will be registered on a techniques course in the first year. Other specialist courses are available in Oxford and at other institutions in the UK, as necessary.

**Supervisor Profile**

Richard Cornall is the Nuffield Professor of Clinical Medicine and the Head of the Nuffield Department of Medicine (NDM). He is a Fellow of the Royal College of Physicians and the Academy of Medical Sciences and Fellow of Magdalen College, Oxford. He is an Honorary Consultant in Renal Medicine.

Over his career he has been interested in using tools from genetics and immunology to study the development and selection of lymphocytes and the causes of autoimmune disease. As a graduate student he generated the first random library of mouse microsatellites markers and played a central role in the first genome-wide linkage analysis of a complex trait, insulin dependent diabetes. At Stanford University, he used animal models to show how the threshold for autoimmunity varied with different combinations of single allele mutations in B cell inhibitory pathways; and he defined the key roles of the syk tyrosine kinase in B cell development and the B cell expression of lymphotoxin development of the T cell zone (with Jason Cyster, UCSF). On his return to Oxford, he described how intracellular self-antigens can positively select B cells, a discovery that has given new insight into understanding SLE, and award-winning work into mechanisms underlying uveitis and vitiligo. Together with Chris Goodnow, he pioneered the use of genome-wide ENU mutagenesis to discover new mechanisms in immune regulation. This led to a series of discoveries in novel genes relevant to human disease, including the autoimmune regulator Roquin, the role of DNA Ligase IV in human SCID, the Themis superfamily and involvement of DOCK8 in maturation of the antibody response and immunological memory. These studies have helped to identify drug targets and to understand human immunodeficiency syndromes caused by hypomorphic mutations in DNA Ligase IV and DOCK8. His laboratory developed ways to screen and identify causative mutations through massively parallel sequencing strategies.

The Lab’s research is in three main areas:

1. B cell selection. We want to understand how the immune system regulates the response to antigens, and how B cells are positively selected and switch during ontogeny.
2. Human Immunodeficiency. We want to understand the mechanisms underlying human immunodeficiency. To achieve this, we are using CRISPR/Cas9 homologous replacement in zygotes and transgenic tools to model a variety of diseases.
3. Modifying the immune response. In collaboration with Simon Davis we have generated humanised mice to study the effectiveness of agonistic and antagonistic antibodies against inhibitory receptors for the treatment of cancer and autoimmune disease. One of our models has been widely used in the pharmaceutical industry for developing anti-cancer PD1 checkpoint Inhibitors.

**Key Publications**

1. Randall KL, Lambe T, Johnson AL, Treanor B, Kucharska E, Domaschenz H, Whittle B, Tze LE, Enders A, Crockford TL, Bouriez-Jones T, Alston D, Cyster JG, Lenardo MJ, Mackay F, Deenick EK, Tangye SG, Chan TD, Camidge T, Brink R, Vinuesa CG, Batista FD, Cornall RJ\*, Goodnow CC\* (\*equal last and corresponding authors). Dock8 mutations cripple B cell immunological synapses, germinal centers and long-lived antibody production. **Nat Immunol** 2009 Dec;10(12):1283-91. *(News and Views:* [*B cell memory: how to start and when to end.*](http://www.ncbi.nlm.nih.gov/pubmed/19915622) *Pelletier N, McHeyzer-Williams MG. Nat Immunol. 10:1233-5, 2009)*
2. Mutation in Fnip1 is associated with B-cell deficiency, cardiomyopathy, and elevated AMPK activity. Siggs OM, Stockenhuber A, Deobagkar-Lele M, Bull KR, Crockford TL, Kingston BL, Crawford G, Anzilotti C, Steeples V, Ghaffari S, Czibik G, Bellahcene M, Watkins H, Ashrafian H, Davies B, Woods A, Carling D, Yavari A, Beutler B, Cornall RJ. **Proc Natl Acad Sci U S A.** 2016 113: E3706-15.
3. [Themis2 lowers the threshold for B cell activation during positive selection.](https://www.ncbi.nlm.nih.gov/pubmed/27992403) Cheng D, Deobagkar-Lele M, Zvezdova E, Choi S, Uehara S, Baup D, Bennett SC, Bull KR, Crockford TL, Ferry H, Warzecha C, Marcellin M, de Peredo AG, Lesourne R, Anzilotti C, \*Love PE, \*Cornall RJ. (\*equal last and corresponding authors) **Nat Immunol** 2017 Feb;18(2):205-213
4. 53BP1 cooperation with the REV7-shieldin complex underpins DNA structure-specific NHEJ. Ghezraoui H, Oliveira C, Becker JR, Bilham K, Moralli D, Anzilotti C, Fischer R, Deobagkar-Lele M, Sanchiz-Calvo M, Fueyo-Marcos E, Bonham S, Kessler BM, Rottenberg S, Cornall RJ, Green CM, Chapman JR. **Nature** 2018 Aug;560(7716):122-127. doi: 10.1038/s41586-018-0362-1. Epub 2018 Jul 25. *(News and Views: Assembling a protective Shield* [*Greenberg*](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Avner%20P%22%5BAuthor%5D) *RA.* [*Nature Cell Biology.*](javascript:AL_get(this,%20'jour',%20'Nature.');) *20:86-863. 2018)*
5. An essential role for the Zn2+ transporter ZIP7 in B cell development. Anzilotti C, Swan DJ, Boisson B, Deobagkar-Lele M, Oliveira C, Chabosseau P, Engelhardt KR, Xu X, Chen R, Alvarez L, Berlinguer-Palmini R, Bull KR, Cawthorne E, Cribbs AP, Crockford TL, Dang TS, Fearn A, Fenech EJ, de Jong SJ, Lagerholm BC, Ma CS, Sims D, van den Berg B, Xu Y, Cant AJ, Kleiner G, Leahy TR, de la Morena MT, Puck JM, Shapiro RS, van der Burg M, Chapman JR, Christianson JC, Davies B, McGrath JA, Przyborski S, Santibanez Koref M, Tangye SG, Werner A, Rutter GA, Padilla-Parra S, Casanova JL, Cornall RJ\*, Conley ME\*, Hambleton S\* (\*equal last and corresponding authors). **Nature Immunol** 20:350-361, 2019. *(News and Views:* [*Revisiting*](http://www.ncbi.nlm.nih.gov/pubmed/19915622) *the old and learning the new of zinc in immunity, Fukada T, Hojyo S, and Takagishi.. Nat Immunol. 20: 248-250, 2019)*.

**Project 3-2**

**Project Title:** B cell activation and selection

**Supervisor:** Professor Richard Cornall - [richard.cornall@ndm.ox.ac.uk](mailto:richard.cornall@ndm.ox.ac.uk)

**Project Overview**

The threshold for activation of the B cell receptor (BCR) determines B cell development, the differentiation of subsets, the response to antigens and immune tolerance. We are beginning to appreciate that the threshold varies through the development of B cells and probably through the lifetime of an individual; but we know little about its molecular basis or how it might be manipulated therapeutically. These are some of the most fundamental questions in immunology with broad implications.

The project is to investigate how the activation threshold of the B cell receptor is set in different B cell subsets and during the immune response to antigens. We will use cellular immunology, gene targeting, and immunoglobulin transgenic models to study these questions, and use ovalbumin (OVA) and hen egg lysozyme (HEL) as model antigens. In recent projects have used similar approaches to study the role of the guanine nucleotide exchange factor DOCK8 and the adaptor protein THEMIS2 in B cells. These studies have highlighted the importance of an antigen’s form and its display on cells in B cell activation, so this is something we would like to explore systematically in more detail. Most studies of B cell activation have used soluble antigens or anti-immunoglobulins to induce B cell activation, whereas most naturally occurring antigens are displayed on membranes.

The first year will be spent developing tools to study the B cell receptor and building models. We will use anti-HEL immunoglobulin transgenic mice to study the response to different forms of HEL or HEL-OVA, and gene targeted cell lines and mice to explore the role of downstream pathways to membrane-bound antigens, starting with the role of Grb2.

Our laboratory is part of the MRC Human Immunology Unit (MRC HIU), in the Weatherall Institute of Molecular Medicine (WIMM) and the Wellcome Trust Centre for Human Genetics (WGH), where we are located. We are well supported with access to cutting edge reagents and technology. There will be several challenging and ground-breaking parts to the project, which will require the student to develop in vitro assays and approaches to the study of rare populations of cells. There will be strong support from the laboratory, supervisors and the wider community in the WIMM, WGH and in Oxford.

The student will study the affected cells at a molecular level, using biochemistry, proteomics and genetics to build and address hypotheses. We expect the study to provide new insight into immune mechanisms and influence the treatment of patients.  Our lab website is at **www.ccmp.ox.ac.uk/cornall-group**.

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Richard Cornall is the Nuffield Professor of Clinical Medicine and the Head of the Nuffield Department of Medicine (NDM). He is a Fellow of the Royal College of Physicians and the Academy of Medical Sciences and Fellow of Magdalen College, Oxford. He is an Honorary Consultant in Renal Medicine.

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3. Modifying the immune response. In collaboration with Simon Davis we have generated humanised mice to study the effectiveness of agonistic and antagonistic antibodies against inhibitory receptors for the treatment of cancer and autoimmune disease. One of our models has been widely used in the pharmaceutical industry for developing anti-cancer PD1 checkpoint Inhibitors.

**Key Publications**

1. [Themis is a member of a new metazoan gene family and is required for the completion of thymocyte positive selection.](http://www.ncbi.nlm.nih.gov/pubmed/19597497) Johnson AL, Aravind L, Shulzhenko N, Morgun A, Choi SY, Crockford TL, Lambe T, Domaschenz H, Kucharska EM, Zheng L, Vinuesa CG, Lenardo MJ, Goodnow CC, Cornall RJ\* and Schwartz RH\* (joint corresponding authors\*). **Nature Immunol** 10:831-9, 2009.  *(News and Views:* [*Themis imposes new law and order on positive selection.*](http://www.ncbi.nlm.nih.gov/pubmed/19621038) *Allen PM. Nat Immunol. 10:805-6, 2009 and Research Highlights:* [*Themis in the thymus*](http://www.nature.com/nature/journal/v460/n7253/full/460309b.html)*. Nature 460: 309-309, 2009)*
2. [Themis2 lowers the threshold for B cell activation during positive selection.](https://www.ncbi.nlm.nih.gov/pubmed/27992403) Cheng D, Deobagkar-Lele M, Zvezdova E, Choi S, Uehara S, Baup D, Bennett SC, Bull KR, Crockford TL, Ferry H, Warzecha C, Marcellin M, de Peredo AG, Lesourne R, Anzilotti C, \*Love PE, \*Cornall RJ. (\*joint corresponding authors) **Nat Immunol** 2017 Feb;18(2):205-213
3. Capturing resting T cells: the perils of PLL. Santos AM, Ponjavic A, Fritzsche M, Fernandes RA, de la Serna JB, Wilcock MJ, Schneider F, Urbančič I, McColl J, Anzilotti C, Ganzinger KA, Aßmann M, Depoil D, Cornall RJ, Dustin ML, Klenerman D, Davis SJ, Eggeling C, Lee SF. **Nat Immunol** 2018 Mar;19(3):203-205. doi: 10.1038/s41590-018-0048-8. Epub 2018 Feb 23
4. 53BP1 cooperation with the REV7-shieldin complex underpins DNA structure-specific NHEJ. Ghezraoui H, Oliveira C, Becker JR, Bilham K, Moralli D, Anzilotti C, Fischer R, Deobagkar-Lele M, Sanchiz-Calvo M, Fueyo-Marcos E, Bonham S, Kessler BM, Rottenberg S, Cornall RJ, Green CM, Chapman JR. **Nature** 2018 Aug;560(7716):122-127. doi: 10.1038/s41586-018-0362-1. Epub 2018 Jul 25. *(News and Views: Assembling a protective Shield* [*Greenberg*](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Avner%20P%22%5BAuthor%5D) *RA.* [*Nature Cell Biology.*](javascript:AL_get(this,%20'jour',%20'Nature.');) *20:86-863. 2018)*
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**Project 4**

**Project Title:** Structural and functional characterisation of the influenza virus nuclear export machinery

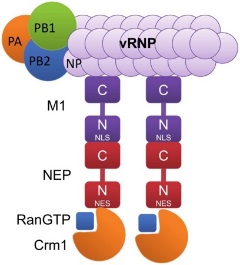
**Supervisor:** Professor Ervin Fodor - [ervin.fodor@path.ox.ac.uk](mailto:ervin.fodor@path.ox.ac.uk)

**Project Overview**

Influenza viruses are important human and animal pathogens; they cause widespread clinical and veterinary disease and have a considerable economic impact. Our laboratories focus on the fundamental molecular mechanisms of influenza virus replication, aiming to understand the molecular determinants of host range and virulence of influenza viruses.

Specifically, our laboratories address questions ranging from how the influenza virus RNA polymerase transcribes and replicates the segmented negative-sense viral RNA genome in the cell nucleus of the infected cell to how the RNA genome is exported from the nucleus and assembles into infectious progeny virus particles. We are also interested in the role of host factors in viral replication as well as in understanding the effects of virus infection on the host cell, the molecular mechanisms of innate immune sensing and host cell responses to viral infection.

The aim of this project is to structurally and functionally characterise the influenza virus nuclear export machinery that is responsible for the nuclear export of viral ribonucleoprotein (RNP) complexes for assembly into virions at the cell membrane. The influenza virus genome consists of eight single stranded negative-sense RNAs that form viral ribonucleoprotein (vRNP) complexes with the viral RNA polymerase (PB1, PB2 and PA) and oligomeric nucleoprotein (NP). vRNPs display a double-helical arrangement resembling a large loop twisted into a helical filament. The RNA polymerase is responsible for replicating the viral RNA in the context of vRNPs in the nucleus of infected cells. The nuclear export of vRNPs is facilitated by the viral factor nuclear export protein (NEP) that mediates the interaction between vRNPs and the cellular export factor Crm1/RanGTP in a viral matrix protein 1 (M1) and viral RNA polymerase dependent manner (see Figure, modified from Paterson and Fodor *PLoS Path* 8(12):e1003019, 2012). However, the structure of NEP and the molecular details of its interaction with vRNPs and Crm1/RanGTP, as well as the role of M1, remain poorly characterised.



This project builds on our recent advances in the structural analysis of the influenza virus RNA polymerase and vRNPs (Fan *et al Nature* 2019 (in press), Serna-Martin *et al Mol Cell* 2018, Hengrung *et al Nature* 2015, York *et al PNAS* 2013) and the development of methods to express and purify mg quantities of NEP (unpublished data). Using NMR we have obtained a preliminary structure of NEP that revealed that its nuclear export signal (NES) is located on an N-terminal *a*-helix (in collaboration with Jason Schnell, Department of Biochemistry, University of Oxford). Moreover, using mass spectrometry, we found a phosphorylation site in NEP, at serine residue 24 (S24), close to the NES, that regulates its interaction with Crm1 (Hutchinson *et al PLoS Path* 2012). Replacement of S24 with a phosphomimetic glutamic acid resulted in NEP forming stable complexes with Crm1/RanGTP (unpublished results).

Initially, we will aim to determine the structure of NEP in complex with Crm1/RanGTP using cryo- EM. However, ultimately we would like to extend these studies to complexes of vRNPs to reveal the atomic details of the interactions of the multiple viral and cellular factors involved. Structural and functional data on the influenza virus NEP and its interaction with viral and cellular factors would greatly expand our limited knowledge of the mechanisms that influenza virus uses to transport its RNA genome out from the host cell nucleus.

**Training opportunities**

The project will employ an inter-disciplinary approach offering training in virology, molecular and cell biology, biochemistry, structural biology (x-ray crystallography, cryo-electron microscopy and small angle X-ray scattering in collaboration with Professor Jonathan Grimes, Division of Structural Biology, University of Oxford), and methods in biophysical characterisation of protein complexes.

**Supervisor**

Professor Ervin Fodor

**Qualifications** DPhil Oxford (1996)

MSc Bratislava (1987)

**Employment history**

2011 – Present Professor of Virology

2010 – 2011 University Lecturer in Virology 2008 – 2010 RCUK Academic Fellow

2002 – 2007 MRC Senior Research Fellow 1998 – 2002 Postdoctoral Research Assistant

*Sir William Dunn School of Pathology, University of Oxford*

1997 – 1998 Postdoctoral Research Associate

*Mount Sinai School of Medicine, New York*

1996 Research Scientist/Wellcome Trust International Research Fellow 1987 – 1991 Research Assistant

*Institute of Virology, Slovak Academy of Sciences, Bratislava*

**Honours, awards, fellowships**

2019 AstraZeneca Award by the Biochemical Society 2008 – 2010 RCUK Academic Fellowship

2002 – 2007 MRC Senior Non-Clinical Research Fellowship

1996 Wellcome Trust International Research Development Fellowship 1997 Max Kade Fellowship

1991-1992 Soros/FCO Scholarship

**Current research grants**

2018 – 2023 MRC Programme Grant (principal investigator)

*“Structure-function relationships of the influenza virus RNA polymerase: influence on virulence, host restriction and innate immune responses”*

2016 – 2019 MRC Project Grant (co-applicant with Professor Achillefs Kapanidis as principal applicant)

“*Single-molecule analysis of the influenza virus transcription and replication mechanisms”*

**CAMS PI**

Professor Tao Deng

**Qualifications** DPhil Oxford (2006)

BSc in Lan Zhou University (1992)

**Employment history**

2010 – Present Professor in Microbiology

*Institute of Pathogen Biology, Chinese Academy of Medical Science (CAMS)*

2009 Senior Scientist (Line manager in virology department)

*National Institute of Biological Standard and Control (NIBSC), London, UK*

2006 – 2008 Postdoctoral Research Scientist

2001 – 2006 Research Assistant

2001 Academic Visitor sponsored by WHO Fellowship.

*Sir William Dunn School of Pathology, University of Oxford, UK*

1992 – 2000 Research Assistant

*Lanzhou Institute of Biological Products (LIBP), Ministry of Public Health, China*

**Honours, awards, fellowships**

2015 – 2018 Distinguished Professor in Peking Union Medical College 2015 Grand Challenges 2015 – Young Scientist Awards

2002 – 2005 Overseas Research Students Awards, UK (ORS)

2005 Chinese Government Award for Outstanding Students Abroad

**Current research grants**

2016 – 2020 CAMS Innovation Fund for Medical Sciences (2016-12M-1-014)

2018 – 2020 National Mega-Project for Infectious Diseases (2018ZX10101001-004) 2019 – 2022 National Natural Science Foundation of China (31070152)

**Key publications**

1. Fan H, Walker AP, Carrique L, Keown JR, Serna Martin I, Karia D, Sharps J, Hengrung N, Pardon E, Steyaert J, Grimes JM and Fodor E (2019) Influenza A virus RNA polymerase structures provide insights into viral genome replication. *Nature* (in press).
2. Dadonaite B, Gilbertson B, Knight M, Trifkovic S, Rockman S, Laederach A, Brown L, Fodor E, Bauer DLV (2019) The structure of the influenza A virus genome. *Nat Microbiol* 2019 Jul 22 (in press).
3. te Velthuis AJW, Long JC, Bauer DLV, Fan RLY, Yen HL, Sharps J, Siegers JY, Killip MJ, French H, Oliva-Martín MJ, Randall RE, de Wit E, van Riel D, Poon LLM, Fodor E (2018) Mini viral RNAs act as innate immune agonists during influenza virus infection. *Nat Microbiol* 3(11):1234-1242.
4. Hengrung N, El Omari K, Serna Martin I, Vreede FT, Cusack S, Rambo RP, Vonrhein C, Bricogne G, Stuart DI, Grimes JM, Fodor E (2015) Crystal structure of the RNA-dependent RNA polymerase from influenza C virus. *Nature* 527:114-117.
5. Zhou Z, Cao M, Guo Y, Zhao L, Wang J, Jia X, Li J, Wang C, Gabriel G, Xue Q, Yi Y, Cui S, Jin Q, Wang J, Deng T (2014) Fragile X mental retardation protein stimulates ribonucleoprotein assembly of influenza A virus. *Nat Commun* 5:32

**Project 5**

**Project Title:** Genomics of host susceptibility to severe infection

**Supervisor:** Professor Julian Knight - [julian@well.ox.ac.uk](mailto:julian@well.ox.ac.uk)

**Project Overview**

This proposal aims to provide training in genomic medicine with particular application to immunology and infectious disease, combining a high-quality scientific research project focused on investigating the 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 recognised to be central to pathogenesis. This occurs only in a very small minority of patients with infections but represents a major burden of disease with sepsis (the dysfunctional response to infection associated with organ dysfunction) being the most common reason for admission to medical intensive care units (ICUs). There are currently few effective treatments for sepsis with a persistently high mortality of 25-30% despite optimal available therapy. This highlights the need for further research in this area to understand why some patients develop a dysregulated response and how knowledge of disease pathogenesis may enable improved treatments. Inherited factors are important, both specifically for sepsis susceptibility and 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 sepsis 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. Recently, 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 related to immune response state and outcome that are robust to source of infectionand 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. 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.

Research plan

1. To identify genetic factors associated with sepsis 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.

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 sepsis and 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 sequencing at the Beijing Genomics Institute with data analysis in Oxford. 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 modular courses established for the Genomic Medicine and Statistics DPhil programme (for which Professor Knight is the Course Director) and through the Medical Sciences Doctoral Training Centre. Clinical training will be enabled through weekly attendance at the Genomic Medicine Multi-Disciplinary Team (chaired by Professor Knight) meetings 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. Further clinical training will be provided in internal medicine through attachment to a clinical firm at the John Radcliffe Hospital under the supervision of Professor Knight (Honorary Consultant) for one month per year.

**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. 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
2. 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.
3. 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.
4. Fairfax BP, Humburg P, Makino S, Naranbhai V, Wong D, Lau E, Jostins L, Plant K, Andrews R, McGee C & Knight JC. 2014 Innate immune activity conditions the effect of regulatory variants upon monocyte gene expression. *Science* **343**: 1246949.
5. 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 doi: 10.1038/s41435-019-0082-z.
6. van der Poll T, van de Veerdonk FL, Scicluna BP & Netea MG. 2017 The immunopathology of sepsis and potential therapeutic targets. *Nat Rev Immunol* **17**: 407-420.

**Project 6**

**Project Title:** Measuring lung inhomogeneity using a novel non-invasive technique to phenotype airways diseases

**Supervisor:** Professor Ian Pavord - [ian.pavord@ndm.ox.ac.uk](mailto:ian.pavord@ndm.ox.ac.uk)

**Project Overview:**

Chronic airways diseases such as asthma and COPD cause significant morbidity and mortality worldwide, with more than 100 million people affected in China. The ability to identify and target “treatable traits” is key in improving clinical outcomes, both in terms of targeting existing therapies to the right phenotype of patient but also in terms of developing new disease-modifying therapies.

A current significant limitation is the lack of sensitive and comprehensive non-invasive physiological tests to detect and monitor disease activity in airways diseases. Spirometry, the mainstay of physiological measurement in airways diseases cannot detect subtle or early disease, particularly disease in the smaller peripheral airways of the lung, is insensitive to treatment effects, is not always concordant with disease control or the presence of underlying inflammation and cannot distinguish different types of pathophysiology.

A recent innovation, developed at the University of Oxford, shows great promise in achieving breakthrough in this area. This non-invasive new technique combines a novel technology that uses laser absorption spectroscopy to provide non-invasive measurements of gas-exchange (with unprecedentent precision), with a mathematical approach to quantify several aspects of inhomogeneity (unevenness) in the lung that relate to lung compliance, blood flow and deadspace. Pilot data suggest the obtained inhomogeneity indices are very repeatable and could provide sensitive early markers of disease activity in the small-airways of the lung.

The overarching aim of the research project will be to use this novel technique, in conjunction with detailed clinical and molecular phenotyping (e.g. blood and sputum eosinophils, cytokines and exhaled nitric oxide), to explore and identify new non-invasive physiological measures of disease activity in airways diseases (asthma and COPD) that will ultimately help us: diagnose disease earlier; better phenotype/stratify patients; predict and monitor disease progression or regression (with treatment) with greater precision and sensitivity.

In COPD, the focus will be to: study chronic smokers – at risk of COPD – but who have normal spirometry to investigate whether lung inhomogeneity measurements are abnormal thus providing early sensitive disease markers. In asthma, the aims will be to investigate whether i. lung inhomogeneity measurements can provide sensitive “early response signals” of treatment effects particularly to new biological therapies; and ii. explore whether on-going eosinophilic inflammation in asymptomatic individuals (with normal spirometry) causes abnormal inhomogeneity measurements in the lung, thus suggesting sub-clinical small-airways disease, that is reversible with targeted treatment.

**Training Opportunities:**

The student will have the opportunity to train in an inter-disciplinary environment that spans clinical respiratory medicine, human physiology and molecular biology, with emphasis on clinical translation. In particular, training opportunities include:

* Gaining of expertise in integrative respiratory physiology, and in particular using a state-of-the-art technology to measure lung function
* Mathematical modelling in analysing gas-exchange measurements
* Statistical analysis including mixed-effect modelling
* Clinical research with human volunteers/patients including Good Clinical Practice Training and submitting ethics board applications
* Opportunity to work in a wet immunology laboratory for some aspects of the work e.g. blood sample and sputum analysis, ELISAs, flow cytometry etc.

**Supervisors:**

**1. Professor Ian Pavord MA DM FRCP FERS FMedSci (Clinical), Professor of Respiratory Medicine, Nuffield Department of Medicine.**

Professor Pavord has a particular interest in asthma and chronic pulmonary disease. His laboratory focuses on exploring underlying inflammatory and immunological processes in airways diseases and translating findings into phenotype-guided treatment, as well as leading clinical trials. He has played a lead role in developing three of the most promising emerging biological treatments in asthma.

**2. Professor Peter Robbins BM BCh MA DPhil (Basic Science, Physiology), Professor of Physiology (Respiratory), Department of Physiology, Anatomy and Genetics.**

Professor Robbins’ is a world’s expert in integrative cardiopulmonary physiology and his laboratory has developed the pioneering technology for gas measurements – as well as the mathematical approach – that will be used in the research project.

**Key publications (up to 5):**

1. Petousi N, Talbot NP, Pavord ID, Robbins PA. **Measuring lung function in airways diseases: current and emerging techniques**. *Thorax* 2019; 74(8): 797-805.
2. Mountain JE, Santer P, ..., Robbins PA. **Potential for noninvasive assessment of lung inhomogeneity using highly precise, highly time-resolved measurements of gas exchange**. *J Appl Physiol* 2018, 124(3): 615-631.
3. Pavord ID et al. **After asthma: redefining airways diseases**. *The Lancet Commissions* 2018, 391 (10118): 350-400.
4. Ciaffoni L, O’Nell DP, …, Robbins PA. **In-airway molecular flow sensing: A new technology for continuous, noninvasive monitoring of oxygen consumption in critical care**. *Sci Adv*. 2016;2(8):e1600560.
5. Haldar P, Brightling CE, …, Pavord ID. **Mepolizumab and exacerbations of refractory eosinophilic asthma**. *New England Journal of Medicine* 2009, 360(10): 973-084.

**Project 7**

**Project Title:** Defining the relationship between DNA repair deficiency, STING and ENPP1

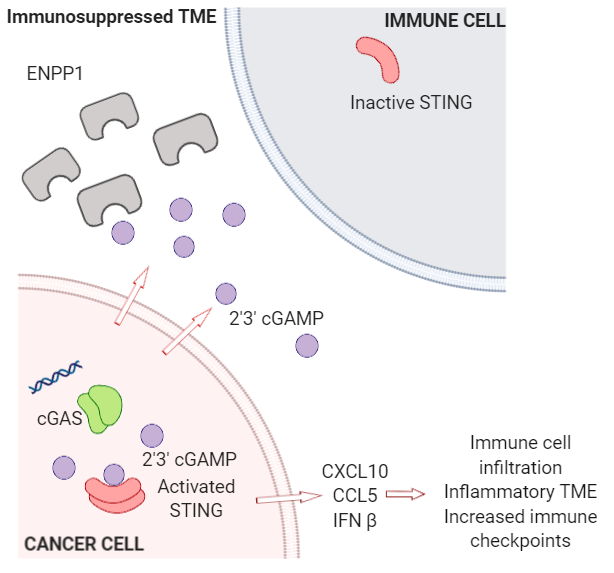
**Supervisor:** Dr Eileen Parkes – [Eileen.parkes@oncology.ox.ac.uk](mailto:Eileen.parkes@oncology.ox.ac.uk)

**Project Overview**

DNA repair deficient tumours are typically associated with an inflamed tumour microenvironment, associated with immunosuppressive mechanisms such as upregulation of immune checkpoints (including PD-L1, CTLA4 and IDO1) and infiltration of macrophages. Previously we demonstrated constitutive activation of the cytosolic DNA-sensing cGAS-STING pathway in tumours deficient in homologous recombination, such as *BRCA*-mutant breast and prostate tumours. However, evidence suggests that STING activation in this context activates pro-tumorigenic mechanisms via non-canonical NFκB signalling downstream of STING, promoting invasion, migration and metastasis. Moreover, high levels of ENPP1 in cGAS-STING high tumours also result in poor prognosis, further suppressing the immune response and associated with an increase in M2 pro-tumorigenic macrophage infiltration. ENPP1 in this context degrades extracellular 2’3’ cGAMP (cGAS-produced STING agonist) preventing acute STING activation in the tumour microenvironment. ENPP1 is now a novel target in immunotherapy, with ENPP1 inhibitors in clinical development. However, currently the relationship between STING activation and production of ENPP1 is not well defined. Detailed characterisation of this relationship will enable the development of effective immunotherapeutic combination strategies with the goal of long-term clinical benefit in DNA repair deficient tumours.

A working model of this relationship is outlined in **Figure 1**. Using novel and existing models of BRCA-deficient solid cancers, the fellow will perform detailed *in vitro* study of the regulation of ENPP1 in tumour cell models and in co-culture with immune models of macrophages. Studies using ENPP1 overexpression vectors will explore the impact of ENPP1 on tumour and immune cell behaviour. Mass spectrometry of ENPP1 immunoprecipitated from DNA repair competent and isogenic repair-deficient models will provide insight into the regulation of ENPP1. Treatment of cGAS and STING wildtype and CRISPR knockout cells (already developed in our group) in vitro with DNA damaging agents and ionising radiation will determine any cGAS-STING dependent resultant upregulation of ENPP1 in the context of DNA damage.

Complementing *in vivo* studies, murine derived tumours, including BRCA2 ko, +/- ionising radiation treatment will be available for analysis of ENPP1 expression and subsequent delineation of the impact on the tumour microenvironment. It is currently not known which cells in the microenvironment contribute to production of ENPP1, and cell sorting of *in vivo* derived tumours will enable analysis of ENPP1 expression. In addition, tissue microarrays of breast and prostate tumours have already been stained for STING, lymphocytes and ENPP1 and will be analysed. Further tissue studies using pre- and post-DNA damaging treatment of oesophageal adenocarcinoma are proposed during this project. Together we anticipate this will provide novel insight into the regulation of ENPP1 in the context of DNA repair deficient cancers, and potential novel approaches to the immunotherapeutic treatment of cancer.



**Figure 1**: In STING active, DNA repair-deficient cancer, aberrant DNA repair results in cytosolic DNA. This DNA is sensed by cGAS, resulting in production of 2’3’ cGAMP. Intrinsic cell production of 2’3’ cGAMP results in STING pathway activation, upregulation of CXCL10, CCL5 and IFNβ, and promotion of an immune cell infiltrate and an inflammatory tumour immune microenvironment. This in turn promotes tumour growth, invasion and metastasis. STING activation results in upregulation of immune checkpoints which is one targetable mechanism of immunosuppression. However, we propose that in addition to this, ENPP1 production is increased, resulting in degradation of cell extrinsic 2’3’ cGAMP, and inability to activate the STING pathway in infiltrating immune cells. In this manner, the tumour benefits from the tumour-promoting effects of inflammation, yet prevents activation of the STING pathway and an anti-tumorigenic response in immune cells.

**Training opportunities**

The fellow will be mentored by Dr Eileen Parkes (Clinician Scientist and Medical Oncologist). They will benefit from the support of the CRUK Cancer Centre’s Clinical Academic Training Programme. This provides:

* A comprehensive specific induction programme. This 2-day event for all new recruits will
* provide students with an overview of the cancer research community in Oxford and the scope for collaborations during their DPhil, the support and training opportunities that are available, and the opportunity to meet and bond as a cohort.
* A structured lecture series during their first year. The syllabus will cover Core Research Skills (including reproducibility and experimental design), Big Data Analytics, Fundamental Cancer Biology, Clinical Cancer Patient Care, and Research for Impact.
* Peer Support via a series of networking events, organised and led by a pair of Student Representatives to give our students the opportunity to broaden their horizons and benefit from each other’s experiences and, additionally, access to a dedicated welfare officer.
* A tailored training and mentorship programme. Each student will have access to mentorship through the Oxford University Hospital Foundation Trust. This will ensure the clinical and academic training needs of the student are maintained throughout their DPhil.

Formal training in CyTOF, RNASeq and bioinformatics will be provided during the fellowship. Training in clinical trial design, set up and execution is also available, through the Oncology Clinical Trials Office and Early Phase Clinical Trials Unit. The fellow will also be able to access seminar series, grand rounds and *ad hoc* lectures relevant to their research training.

Clinical observerships will be established, according to the fellow’s interests, at the Oxford Cancer Centre of the Oxford University Hospitals NHS Foundation Trust. These will provide the opportunity to engage in 1:1 teaching on specialist clinical topics.

***Supervisor***

Eileen Parkes is a medical oncologist, clinician scientist and junior group leader at the University of Oxford. She initially identified STING activation in the context of BRCA-deficient breast cancer, and has developed novel models of BRCA-deficient tumours within her lab. Her clinical interest is in novel immunotherapeutic agents, combination immunotherapy regimens and the treatment of upper GI cancers. Her work is focused on the regulation and determination of anti- and pro-tumorigenic effects of the STING innate immune pathway, with an interest on the consequences of STING pathway activation on the tumour microenvironment. As a member of her research group at the University of Oxford the fellow will benefit from training in molecular biology techniques with an emphasis on the clinical translation of their findings.

**Key publications**

1. Sharma P, Barlow WE, Godwin AK, **Parkes EE**, Knight LA, Walker SM, Kennedy RD, Harkin DP, Logan GE, Steele CJ, Badve S, Gokmen-Polar Y, Pathak HB, Isakova K, Linden HM, Porter P, Pusztai L, Thompson A, Tripathy DT, Hortobagyi GN, Hayes DF. Validation of a DNA Damage Response Immune Response Assay in Triple Negative Breast Cancer (TNBC) Patients from the SWOG 9313c Trial. *In press: Journal of Clinical Oncology,* August 2019.
2. **Parkes EE**,Walker SM, Taggart LE, McCabe N, Knight L, McCloskey KD, Buckley NE, Savage KI, Salto-Tellez M, McQuaid S, Harte MT, Mullan PB, Harkin DP, Kennedy RD. Activation of STING-dependent innate immune signalling by S-phase specific DNA damage in breast cancer. *Journal of the National Cancer Institute* 2017; 109(1).
3. Wilkinson R, McCabe N, **Parkes EE**, Barros E, Johnston D, Ali R, Lappin K, Greenberg R, Harkin PD, McIntosh S, Kennedy RD, Savage KI. Topoisomerase II inhibitors induce cGAS-STING dependent inflammation resulting in cytokine induction and immune checkpoint activation. *Under review: Clinical Cancer Research*
4. Turkington R, Knight LA, Blayney JK, Secrier M, Douglas R, **Parkes EE**, Sutton EK, Stevenson L, McManus D, Halliday S, McCavigan AM, Logan GE, Walker SM, Steele CJ, Perner J, Bornschein J, MacRae S, Miremadi A, McCarron E, McQuaid S, Arthur K, James J, Eatock M, O’Neill R, Noble F, Underwood TJ, Harkin DP, Salto-Tellez M, Fitzgerald R, Kennedy RD. Immune activation by DNA damage predicts response to chemotherapy and survival in oesophageal adenocarcinoma. *Gut,* 2019. doi: 10.1136/gutjnl-2018-317624.
5. Humphries M, Hynes S, Bingham V, Cougot D, James JA, Patell-Socha F, **Parkes EE**, O’Rorke M, Irwin GW, McArt D, Kennedy RD, Mullan P, McQuaid S, Salto-Tellez M, Buckley N, Blayney JK. Automated tumour recognition and digital pathology scoring unravels new role for PD-L1 in predicting good outcome in ER-/HER2+ breast cancer. *Journal of Oncology,* 2018. doi: 10.1155/2018/2937012

**Project 8-1**

**Project Title:** Characterising the spatial relationships of the immune infiltrate in pancreatic cancer

**Supervisor:** Professor Mark Middleton - [mark.middleton@oncology.ox.ac.uk](mailto:mark.middleton@oncology.ox.ac.uk)

**Project Overview**

Pancreatic ductal adenocarcinoma (PDAC) has a 5-year survival rate of 4% - this figure, the worst of any human cancer, has not changed in over 50 years. Surgery offers the only chance of cure when the cancer is caught at its earliest stage (T1 N0 MO), though the 5-year survival rate is still poor at 40%. The genetics of pancreatic cancer are well described. Driver mutations are observed in the *KRAS* oncogene, which is currently considered an ‘undruggable’ gene. Additional tumour suppressors such as *p53, p16* and SMAD4 are also affected. A small fraction of the cancer is tumour tissue, whilst the majority is the microenvironment, which consists of immune infiltrate, stroma and a desmoplastic collagen reaction. We have used an integrative analysis of four different PDAC gene expression studies to derive a consensus immune PDAC classification. We found adaptive, innate and immune-exclusion immunologic signatures across different PDAC subtypes, which are also prognostic in independent cohorts

The fellow will perform deep tissue profiling using a multiplex imaging machine, the CODEX, a technology that uses unique DNA tags as a means of iteratively measuring more than 40 parameters within the same tissue. More than 40 human antibodies have been validated using this approach, including numerous immune markers, checkpoint ligands, tumour markers and cellular activity markers. By measuring 40 simultaneous markers within the same tissue, CODEX has the potential greatly to enhance our knowledge of the tumour microenvironment and more accurately define immune infiltrates at the single cell level.

The work will complement existing research with T cell CyTOF, innate CyTOF and single cell sequencing in the same tumours. Initial work will focus on tumours (n=50) taken from untreated patients. In the second half of the fellowship tumours will come from patients treated with chemo- and/or immune-therapy pre-operatively (n=30) to understand how therapeutic stress shapes immune cell interactions in the pancreas.

**Training opportunities**

The fellow will be mentored by Professor Middleton (Director of Oncology) and Dr Sivakumar (Clinician Scientist). He/she will benefit from the support of the CRUK Cancer Centre’s Clinical Academic Training Programme. This provides:

* A comprehensive specific induction programme. This 2-day event for all new recruits will provide students with an overview of the cancer research community in Oxford and the scope for collaborations during their DPhil, the support and training opportunities that are available, and the opportunity to meet and bond as a cohort.
* A structured lecture series during their first year. The syllabus will cover Core Research Skills (including reproducibility and experimental design), Big Data Analytics, Fundamental Cancer Biology, Clinical Cancer Patient Care, and Research for Impact.
* A two-tier Peer Support scheme. Firstly, a series of networking events, organised and led by a pair of Student Representatives to give our students the opportunity to broaden their horizons and benefit from each other’s experiences and, second, access to a dedicated welfare officer.
* A tailored training and mentorship programme. Each student will have access to mentorship through the Oxford University Hospital Foundation Trust. This will ensure the clinical and academic training needs of the student are maintained throughout their DPhil.

Formal training in CODEX, image analysis and bioinformatics will be provided during the fellowship. Training in clinical trial design, set up and execution is also available, through the Oncology Clinical Trials Office and Early Phase Clinical Trials Unit. The fellow will also be able to access seminar series, grand rounds and *ad hoc* lectures relevant to their research training.

Clinical observerships will be established, according to the fellow’s interests, at the Oxford Cancer Centre of the Oxford University Hospitals NHS Foundation Trust. These will provide the opportunity to engage in 1:1 teaching on specialist clinical topics.

**Supervisor**

Mark Middleton is Professor of Experimental Cancer Medicine at the University of Oxford, where he founded the Early Phase Clinical Trials Unit. Co-Director of the Cancer Research UK Oxford Centre, he is the lead for its Clinical Academic Training Programme. His clinical interests are in drug development and in the treatment of upper GI cancers and melanoma. His research work focuses on the characterisation of patients and their cancer and on the mechanisms that determine the outcomes of DNA damaging and immune therapies. He has been Chief or Principal Investigator on over 100 trials and has been closely involved in the clinical development of several new therapies including DNA repair inhibitors, MEK inhibitors, immune checkpoint inhibitors and ImmTACs.

**Key publications**

1. Immunophenotypes of pancreatic ductal adenocarcinoma: meta-analysis of transcriptional subtypes (2019). de Santiago I, Yau C, Heij L, Middleton MR, Markowetz F, Grabsch HI, Dustin ML, Sivakumar S. Int J Cancer 145(4):1125-1137.
2. A phase 1 study to assess the safety, tolerability, and pharmacokinetics of CXD101 in patients with advanced cancer (2019). Eyre TA, Collins GP, Gupta A, Coupe N, Sheikh S, Whittaker J, Wang LM, Campo L, Soilleux E, Tysoe F, Cousins R, La Thangue N, Folkes LK, Stratford MRL, Kerr D, Middleton MR. Cancer. 125(1):99-108.
3. Safety and feasibility of ultrasound-triggered targeted drug delivery of doxorubicin from thermosensitive liposomes in liver tumours (TARDOX): a single-centre, open-label, phase 1 trial (2018). Lyon PC, Gray MD, Mannaris C, Folkes LK, Stratford M, Campo L, Chung DYF, Scott S, Anderson M, Goldin R, Carlisle R, Wu F, Middleton MR, Gleeson FV, Coussios CC. Lancet Oncol. 19(8):1027-1039.
4. Differential clonal evolution in oesophageal cancers in response to neo-adjuvant chemotherapy (2016). Findlay JM, Castro-Giner F, Makino S, Rayner E, Kartsonaki C, Cross W, Kovac M, Ulahannan D, Palles C, Gillies RS, MacGregor TP, Church D, Maynard ND, Buffa F, Cazier JB, Graham TA, Wang LM, Sharma RA, Middleton M, Tomlinson I. Nat Commun. 7:11111. doi: 10.1038/ncomms11111.
5. Pancreatic cancer hENT1 expression and survival from gemcitabine in patients from the ESPAC-3 trial (2014). Greenhalf W, Ghaneh P, Neoptolemos JP, Palmer DH, Cox TF, Lamb RF, Garner E, Campbell F, Mackey JR, Costello E, Moore MJ, Valle JW, McDonald AC, Carter R, Tebbutt NC, Goldstein D, Shannon J, Dervenis C, Glimelius B, Deakin M, Charnley RM, Lacaine F, Scarfe AG, Middleton MR, Anthoney A, Halloran CM, Mayerle J, Oláh A, Jackson R, Rawcliffe CL, Scarpa A, Bassi C, Büchler MW; European Study Group for Pancreatic Cancer. J Natl Cancer Inst. 106(1):djt347.

**Project 8-2**

**Project Title:** Describing peripheral T cell responses to immunotherapy

**Supervisor:** Professor Mark Middleton - [mark.middleton@oncology.ox.ac.uk](mailto:mark.middleton@oncology.ox.ac.uk)

**Project Overview**

Checkpoint inhibitor treatment has revolutionised treatment for cancers such as malignant melanoma in recent years, producing durable responses in patients with metastatic malignancy. Despite this progress, responses are still only seen in a subset of patients and the phenotype and specificity of the T cells responsible for determining efficacy remain poorly characterised. A better understanding of T cell behaviour during immunotherapy treatment is required to identify markers of response and resistance and to predict effective combination strategies. We have performed high-dimensional single cell analysis by mass cytometry of serial peripheral blood mononuclear cells from metastatic melanoma patients before and during combination anti-CTLA4/anti-PD1 treatment or anti-PD1 monotherapy. Tetramers loaded with a pool of tumour-associated and microbial peptides were used to track antigen-specific responses over time and fluorescence activated cell sorting and cloning performed for selected patients to establish tumour specific T cell clones at different time points during immunotherapy treatment. We observe the accumulation of activated effector CD8+ T cells in response to treatment, more pronounced with combination anti-CTLA4/anti-PD1 treatment than anti-PD-1 monotherapy. Preliminary data indicate these activated cells to be enriched in tumour-specific compared to microbial-specific T cells with diversification and/or expansion of the tumour but not microbial-specific T cell response observed over the course of treatment. This was accompanied by transient accumulation of T cell clones with altered functional avidity for certain common tumour-associated antigens such as MART1. This integrated immune monitoring approach demonstrates that it is feasible to rapidly (4-6 weeks) reconstruct *in vitro* the patient-specific repertoire of circulating tumour-specific T cells mobilized in response to immunotherapy.

The fellow will explore these observations further by analysing T cell responses to checkpoint inhibitor immunotherapy across tumour types (non-small cell lung cancer, oesophageal cancer and melanoma) and extend the analysis to novel immunotherapy agents (IMCgp100 and stimulators of innate immunity). Single cell RNA-seq will be used to confirm transient activation and persistence of specific clonotypes, and to describe the transcriptional profile of tumour-specific T cells during therapy. The specificity and functional avidity of tumour-specific T cell clones will be assessed by intracellular cytokine staining, and their TCR usage through RNA sequencing. Single cell RNA-sequencing of ex-vivo sorted tumour tetramer positive cells will be used to track transcriptional changes over the course of treatment within individual T cell clones. This will provide insights into the mechanisms underpinning effective immunotherapy, and identify T cells and TCR clonotypes with maximum therapeutic potential to guide subsequent personalised adoptive cell therapies or vaccination strategies.

**Training opportunities**

The fellow will be mentored by Professor Middleton (Director of Oncology) and Dr Woodcock (Clinician Scientist). He/she will benefit from the support of the CRUK Cancer Centre’s Clinical Academic Training Programme. This provides:

* A comprehensive specific induction programme. This 2-day event for all new recruits willprovide students with an overview of the cancer research community in Oxford and the scope for collaborations during their DPhil, the support and training opportunities that are available, and the opportunity to meet and bond as a cohort.
* A structured lecture series during their first year. The syllabus will cover Core Research Skills (including reproducibility and experimental design), Big Data Analytics, Fundamental Cancer Biology, Clinical Cancer Patient Care, and Research for Impact.
* A two-tier Peer Support scheme. Firstly, a series of networking events, organised and led by a pair of Student Representatives to give our students the opportunity to broaden their horizons and benefit from each other’s experiences and, second, access to a dedicated welfare officer.
* A tailored training and mentorship programme. Each student will have access to mentorship through the Oxford University Hospital Foundation Trust. This will ensure the clinical and academic training needs of the student are maintained throughout their DPhil.

Formal training in CyTOF, RNASeq and bioinformatics will be provided during the fellowship. Training in clinical trial design, set up and execution is also available, through the Oncology Clinical Trials Office and Early Phase Clinical Trials Unit. The fellow will also be able to access seminar series, grand rounds and *ad hoc* lectures relevant to their research training.

Clinical observerships will be established, according to the fellow’s interests, at the Oxford Cancer Centre of the Oxford University Hospitals NHS Foundation Trust. These will provide the opportunity to engage in 1:1 teaching on specialist clinical topics.

**Supervisor**

Mark Middleton is Professor of Experimental Cancer Medicine at the University of Oxford, where he founded the Early Phase Clinical Trials Unit. Co-Director of the Cancer Research UK Oxford Centre, he is the lead for its Clinical Academic Training Programme. His clinical interests are in drug development and in the treatment of upper GI cancers and melanoma. His research work focuses on the characterisation of patients and their cancer and on the mechanisms that determine the outcomes of DNA damaging and immune therapies. He has been Chief or Principal Investigator on over 100 trials and has been closely involved in the clinical development of several new therapies including DNA repair inhibitors, MEK inhibitors, immune checkpoint inhibitors and ImmTACs.

**Key publications**

1. Immunophenotypes of pancreatic ductal adenocarcinoma: meta-analysis of transcriptional subtypes (2019). de Santiago I, Yau C, Heij L, Middleton MR, Markowetz F, Grabsch HI, Dustin ML, Sivakumar S. Int J Cancer 145(4):1125-1137.
2. Relationship between clinical efficacy and AEs of IMCgp100, a novel bispecific TCR–anti-CD3, in patients with advanced melanoma (2019). Mark R. Middleton, Neil Steven, TR Jeffry Evans, Jeffrey Infante, Mario Sznol, Omid Hamid, Alexander Shoushtari, Alan Anthoney, Avinash Gupta, Victoria K Woodcock, Rachael Easton, Philippa Corrie. DOI: 10.1200/JCO.2019.37.15\_suppl.9530 Journal of Clinical Oncology 37, no. 15\_suppl (May 20, 2019) 9530-9530.
3. Characterisation of the changing genomic landscape of metastatic melanoma using cell free DNA (2017). Cutts A, Venn O, Dilthey A, Gupta A, Vavoulis D, Dreau H, Middleton M, McVean G, Taylor JC, Schuh A. NPJ Genom Med. 2:25. doi: 10.1038/s41525-017-0030-7.
4. Differential clonal evolution in oesophageal cancers in response to neo-adjuvant chemotherapy (2016). Findlay JM, Castro-Giner F, Makino S, Rayner E, Kartsonaki C, Cross W, Kovac M, Ulahannan D, Palles C, Gillies RS, MacGregor TP, Church D, Maynard ND, Buffa F, Cazier JB, Graham TA, Wang LM, Sharma RA, Middleton M, Tomlinson I. Nat Commun. 7:11111. doi: 10.1038/ncomms11111.
5. NY-ESO-1 specific antibody and cellular responses in melanoma patients primed with NY-ESO-1 protein in ISCOMATRIX and boosted with recombinant NY-ESO-1 fowlpox virus. (2014) Chen JL, Dawoodji A, Tarlton A, Gnjatic S, Tajar A, Karydis I, Browning J, Pratap S, Verfaille C, Venhaus RR, Pan L, Altman DG, Cebon JS, Old LL, Nathan P, Ottensmeier C, Middleton M, Cerundolo V. Int J Cancer. doi: 10.1002/ijc.29118.

**Project 9**

**Project Title:** Understanding host pathways that regulate hepatitis B virus to design new curative strategies

**Supervisor:** Professor Jane McKeating - [jane.mckeating@ndm.ox.ac.uk](mailto:jane.mckeating@ndm.ox.ac.uk)

**Project Overview**

Hepatitis B virus (HBV) is a major health problem, with more than 240 million people chronically infected and 780,000 deaths/year from HBV-related liver diseases such as cirrhosis and hepatocellular carcinoma. Currently available treatments suppress viral replication but are not curative, due to the persistence of viral covalently closed circular DNA (cccDNA) genome in the liver. HBV cccDNA is frequently referred to as a viral mini-chromosome, where gene transcription is regulated by DNA methylation and epigenetic modifications. Our understanding of the cccDNA epigenome in the liver is limited, providing a barrier to the development of effective cures. The aim of our laboratory is to define host pathways that regulate HBV replication and to realize the impact of hepatocellular differentiation status on cccDNA genesis and transcriptional activity. Our ultimate goal is to uncover host pathways that provide new targets for anti-viral intervention. Here we propose two projects that relate to this central theme.

In the first project we will use state-of-art model systems including iPSc-derived hepatocytes combined with recent minicircle HBV technology to study the effect of cellular differentiation status on the HBV cccDNA genesis, epigenome and transcriptional activity. All of the techniques iPSc cell differentiation, cccDNA chromatin immunoprecipitation and PCR methodology to quantify viral transcripts are available for this project. Ongoing studies in our laboratory highlight a role for hypoxia inducible factors (HIFs) to bind cccDNA and activate viral transcription. The recent availability of HIF inhibitors provide novel anti-viral agents and the student would join a team who are establishing novel *in vitro* systems that utilize physiological oxygen tensions to study HBV replication. We hypothesise that targeted disruption of key host transcription factors will provide novel strategies to regulate HBV replication and may provide a novel therapeutic strategy in humans.

In the second project we will study the role of HBV integrants and their role in driving surface glycoprotein (HBsAg) expression in chronic disease. High HBsAg levels have been associated with limited or weak T cell responses and deletion of T cells recognizing specific epitopes, suggesting that persistent HBsAg expression may drive HBV-specific T cell exhaustion. We will evaluate the mechanistic role of HBsAg as an immune modulator using available T cell clones targeting different HBV antigens and integrant model systems. Data from our laboratory show that the majority of chronic HBV infected subjects express high levels of HBsAg that are derived from integrated viral genomes. Preliminary analysis of selected cases shows a high frequency of HBsAg+ hepatocytes in the liver, consistent with a clonal expansion of hepatocytes bearing viral integrants, raising potential concerns for HBsAg reactive T-cell therapy and associated risk of inducing fulminant hepatitis. These observations have obvious implications for our understanding of HBV biology and immune based therapies. High content multiplex imaging systems provide exciting opportunities to study viral antigen expressing hepatocytes and their relationship to immune cells in the liver. This project aims to define the role of HBsAg in regulating HBV specific T cell exhaustion and will form part of a programme to develop immune based therapeutic strategies to control HBV and associated liver cancer.

**Supervisors**

Primary: Professor Jane McKeating

Secondary: Professor Tao Dong.

**Supervisor’s short profile and links to web profile**

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 HCV entry into the liver and pathways for viral dissemination. An important aspect of their current programme is to understand the basic biology of HBV and to discover host pathways that regulate viral replication and to use this knowledge to design new targeted anti-viral strategies. Jane has published over 180 research papers on HIV, hepatitis B and C viruses and that have received over 16,000 citations (Current H-index of 68 – SCI).

<https://www.ndmrb.ox.ac.uk/team/jane-mckeating>

<https://www.ndm.ox.ac.uk/principal-investigators/researcher/jane-mckeating>

Tao Dong has held the post of Professor of Immunology in the MRC Human Immunology Unit at Oxford University since 2014. For the past two decades, Tao studied qualitative changes in HIV-specific cytotoxic T cells associated with HIV disease progression, she then expanded her research interests to include work on influenza virus infection. In 2010 she became the Head of the human anti-viral 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. Tao has published 209 peer-reviewed journal papers and has achieved a H-index of 51 (google scholar).

<https://www.ndm.ox.ac.uk/principal-investigators/researcher/tao-dong>

**Key publications:**

1. ABD HAMID M *et al*, (2019). Enriched HLA-E and CD94/NKG2A interaction limits antitumor CD8+ tumor-infiltrating T lymphocyte responses. Cancer Immunol Res **7**: 1293-1306.
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.
3. Wing PAC, Davenne T, Wettengel J, Lai AG, Chakraborty A, *et al.* 2019. A dual role for SAMDH1 in HBV cccDNA synthesis and RT-dependent particle genesis. Life Science Alliance, *in press.*
4. ZHANG C, PENG Y, HUBLITZ P, ZHANG H, DONG T. 2018. Genetic abrogation of immune checkpoints in antigen specific cytotoxic T-lymphocyte as a potential alternative to blockade immunotherapy. Sci Rep **8:** 5549
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.
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.
7. ZHANG Y-H, ZHAO Y, LI N, PENG Y-C, GIANNOULATOU E, JIN R-H, YAN H-P, WU H, LIU J-H, LIU N, WANG D-Y, SHU Y-L, HO L-P, KELLAM P, MCMICHAEL A, DONG T. 2013. Interferon-induced transmembrane protein-3 genetic variant rs12252-C is associated with severe influenza in Chinese individuals. Nat Commun **4**: 1418.
8. LEE LY-H, HA DLA, SIMMONS C, DE JONG MD, CHAU NVV, SCHUMACHER R, PENG YC, MCMICHAEL AJ, FARRAR JJ, SMITH GL, TOWNSEND ARM, ASKONAS BA, ROWLAND-JONES S, DONG T. 2008. Memory T cells established by seasonal human influenza A infection cross-react with avian influenza A (H5N1) in healthy individuals. J Clin Invest, **118**: 3478-3490.

**Project 10-1**

**Project Title:** Self-management of chronic kidney disease through M-health

**Supervisor:** Professor Lisa White - [lisa.white@ndm.ox.ac.uk](mailto:lisa.white@ndm.ox.ac.uk)

**Project Overview**

Chronic kidney disease (CKD) is increasingly recognised as a global public health problem, with the burden of CKD rising worldwide and rising fastest in low-income and middle-income countries. Among other pressing issues, it important to empower patients in the self-management of their condition (*1*). There are many features of the condition which can be effectively self-managed including: medication reminders; dietary supplements; lifestyle changes (diet, exercise, stress-reduction, mindfulness etc); recording of symptoms and treatment side effects; blood pressure and other key measurements; accessing current medical information in appropriate formats; communication with other patients; communication with western, traditional and complementary health practitioners. Although it is technically possible, a user-friendly M-health application to support all these activities is not yet available.

**Aims and Objectives**

The aim of this project is to develop a prototype smartphone application for the holistic self-management of chronic kidney disease.

The objectives are:

1. Develop an inclusive list of all self-management activities of CKD carried out by patients
2. Explore all currently available and upcoming technologies for home monitoring of CKD and how they interface with smartphone technology
3. Establish links with nephrology research departments in UK, China and internationally to design an appropriate M-Health application prototype
4. Establish links and collaborate with application developers within Oxford University and beyond to deliver the prototype
5. Work with nephrology research departments to develop a trial design for the prototype

**Person specification**

This project would be most suited to a candidate with experience in nephrology and/or smartphone health application development.

**Training Opportunities**

The successful candidate will be provided with a bespoke training package with online and residential courses to support their research project and fill in knowledge gaps designed in partnership with Professor Lisa White.

**Publications:**

1. A. Levin *et al.*, Global kidney health 2017 and beyond: a roadmap for closing gaps in care, research, and policy. *Lancet* **390**, 1888-1917 (2017).

**Project 10-2**

**Project Title:** Modelling vaccine hesitancy and strategies to address it

**Supervisor:** Professor Lisa White - [lisa.white@ndm.ox.ac.uk](mailto:lisa.white@ndm.ox.ac.uk)

**Project Overview**

Vaccine hesitancy is becoming a major public health issue, which could dramatically reduce the impact of current and future vaccines. The World Health Organization lists vaccine hesitancy among their ten threats to global health in 2019. The drivers of vaccine uptake and hesitancy are diverse and multiple (*1, 2*). Modelling frameworks to consider this new public health risk must be well informed by the sociological, economic and political drivers.

**Aims and Objectives**

The aim of this project is to use a mixed methods approach to understand and develop strategies to address vaccine hesitancy.

The objectives are:

1. Interrogate the way that adverse events and vaccine failures are costed compared to morbidity and mortality from the infection
2. Take a multi-species approach to the problem, for example consider the impact of dengue vaccine-induced deaths on measles vaccine coverage.
3. Use modelling informed by qualitative social sciences research to explore the dynamic interplay between disease infection rates, vaccine coverage and vaccine hesitancy.
4. Consider the behaviour of hesitancy as in infectious idea and use viral marketing concepts to understand it and how to minimise it.

**Person specification**

This project would be most suited to a candidate with experience in the clinical and/or social context of vaccine hesitancy. Economic and mathematical modelling expertise would also be desirable.

**Training Opportunities**

The successful candidate will be provided with a bespoke training package with online and residential courses to support their research project and fill in knowledge gaps designed in partnership with Professor Lisa White.

**Publications:**

1. K. Attwell, D. T. Smith, Hearts, minds, nudges and shoves: (How) can we mobilise communities for vaccination in a marketised society? *Vaccine* **36**, 6506-6508 (2018).
2. B. Han *et al.*, Has the public lost confidence in vaccines because of a vaccine scandal in China. *Vaccine* **37**, 5270-5275 (2019).

**Project 11**

**Project Title:** Mechanical guidance of anti-tumour responses of metabolically energetic T cells

**Supervisor:** Dr Marco Fritzsche - [marco.fritzsche@kennedy.ox.ac.uk](mailto:marco.fritzsche@kennedy.ox.ac.uk)

**Project Overview**

Biomedical sciences increasingly recognise the importance of mechanobiology in health and disease. While most mechanisms of the human immune response are adequately explained by cell-biology, bio-chemistry, and genetics, many of its features profoundly depend on biomechanical features. One such scenario involves the ability of metabolically energetic T cells to differently respond to tumours expressing mechanics-associated signalling molecules, highlighting additional parameters needed to fully explain anti-tumour responses. A pivotal event promoting these responses is binding of antigens to antigen receptors and subsequent actin cytoskeleton reorganisations resulting in intracellular signalling and immunological synapse formation. The IS is a specialised interface at the cell-cell contact between lymphocytes and antigen presenting cells (APCs), which has been unleashed to enhance T-cell function in cancer immunotherapy. T-cell receptor (TCR) mediated activation of T cells by APCs depends on their actin cytoskeleton, which is primary determinant of T-cell biomechanics.

We have recently demonstrated that immune T cells dynamically adjust their biomechanics to facilitate antigen recognition. Specifically, we further found that mechanics-associating engagement of E-Cadherin+ cancer cells by CD103+ T cells mediate enhanced T-cell mobility, tumour clustering, killing of cancer tumour cells. This research project aims to elucidate the dynamic interplay of tissue mechanics and antigen recognition in the context of mechanical signalling and mechanosensation in T cells interacting with cancer tumours. Further, we make use of advanced super-resolution microscopy and high-throughput light-sheet microscopy. Single molecule based biophysical analysis tools will enable us to study the biomechanics in activating T cells. We will initially investigate the dynamics of the above detailed players in Jurkat T-cells interacting with protein-functionalised glass surfaces and then extend our studies to primary lymphocyte clones specific to antigens with varying affinities interacting with tumour reconstituting assays.

**Training opportunities**

The candidate will be working in the Fritzsche and Dong laboratories based at the Kennedy Institute of Rheumatology (KIR) and Weatherall Institute for Molecular Medicine and at the University of Oxford, world-leading centres in the fields of immunology with a strong emphasis on clinical translation. Throughout the studentship, we will encourage the candidate to attend weekly seminars at the Institute given by global leaders in the fields of immunology and biophysics. The DPhil candidate will benefit from combined supervision by a biophysicist with expertise in advanced microscopy as well as molecular immunologist with great expertise with T-cell clones. Weekly lab meetings, where students and post-doctoral scientists discuss their findings will allow the candidate to gain invaluable research experience, manuscript publication, and conference presentations. There is support available from post-doctoral scientists in our groups and laboratory managers to become proficient in cell and molecular biological techniques, advanced biophysical and microscopy techniques including high-throughput lightsheet microscopy, tissue culture, and force probing systems. Overall, the CSC-CAMS-Oxford scholarship provides an ideal platform for intellectual development and translational research**.**

**Supervisor**

Dr. Marco Fritzsche leads the Biophysical Immunology group within the the Kennedy Institute for Rheumatology and the Human Immunology Unit at the Weatherall Institute for Molecular Medicine at the University of Oxford. The BPI lab develops and employs novel custom-designed technology at the interface of biophysics and immunology to investigate the impact of mechanobiology in health and disease. Dr. Fritzsche holds a MSc in theoretical physics, and conducted his PhD in experimental biophysics and cell-biology at the London Centre for Nanotechnology at the University College London, UK. He performed his Postdoctoral work at the University of Oxford in close collaboration with the Howard Hughes Medical Institute Janelia Research Campus, USA.

**CAMS PI**

Prof 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 since 2016.

She 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 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.

**Key publications.**

1. Colin-York et al, Cytoskeletal Control of Antigen-Dependent T Cell Activation, Cell Reports, 2019.
2. Colin-York et al, Spatiotemporally Super-resolved Volumetric Traction Force Microscopy, Nano Letters, 2019.
3. Hamid et al, Efficient anti-tumor effector responses by metabolically energetic CD103 + T cells with accelerated T cell apoptosis, Cancer Immunology Research, 2019.

**Project 12**

**Project Title:** Understanding how epigenetic marks are established

**Supervisor:** Professor Richard Gibbons - [richard.gibbons@imm.ox.ac.uk](mailto:Richard.gibbons@imm.ox.ac.uk)

**Project Overview**

The correct regulation of gene expression is critical for normal mammalian development and this depends on the epigenetic landscape of the genome in which DNA methylation plays a central role. Despite having identified key proteins of the methylation machinery we still do not understand how DNA methylation is actually established. Aberrant DNA methylation is a feature of cancer and understanding this process has far reaching implications. One approach to understanding the molecular cues that trigger DNA methylation is to study proteins known to affect DNA methylation patterns such as the chromatin remodelling protein ATRX.

We have previously shown that germline ATRX mutations in human and mouse lead to widespread changes in global repetitive DNA methylation and more recently abnormal methylation in normally unmethylated CpG islands has been observed. This appears to occur at the very earliest stages of development and we have recapitulated the phenomenon in human embryonic stem cells in which we have mutated ATRX. Concentrating on a particular locus we have shown that the propensity for an allele to become methylated is associated with the presence of a G-rich repeat, to which ATRX normally binds and which has the potential to form a non-linear DNA structure called a G-quadruplex (G4). An intriguing observation is that the allele with the longer repeat is always preferentially methylated over the smaller allele leading to allele specific differences in gene expression. In alleles that are not methylated gene expression is still down regulated indicating that additional epigenetic features are involved. We have now developed an inducible model whereby expression of ATRX can be turned off and the sequence of events leading to DNA methylation can be observed. This system offers invaluable insights into the *cis* and *trans*-acting factors involved in the process of *de novo* DNA methylation.

**Training Opportunities**

The student will learn to genetically manipulate human stem cells and induced pluripotent stem cells using CRISPR/Cas9. The epigenetic profile will be determined by ChIP for histone modifications and bisulphite sequencing for DNA methylation. The proteomics of the loci studied will be determined using a new technique combining CRISPR based genome targeting, affinity labelling and quantitative proteomics. Gene expression will be studied both in bulk and at single cell basis using 10x genomics technology and next generation sequencing.

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. Students are also able to attend the Methods and Techniques course run by the MRC Weatherall Institute of Molecular Medicine. This course runs through the year, ensuring that students have the opportunity to build a broad-based understanding of differing research techniques.

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.

The 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 support the careers of female students and staff.

**Supervisors**

**Richard Gibbons** (BM BCh, DPhil, FRCP, FMedSci) qualified in Medicine at Oxford Medical School in 1986 and is currently Professor of Clinical Genetics at the University of Oxford, Honorary Consultant in Clinical Genetics and a Principal Investigator in the MRC Molecular Haematology Unit at the Weatherall Institute of Molecular Medicine. The main aim of his lab is to understand the role of a chromatin remodelling protein, ATRX, in transcriptional regulation and how, when mutated, it leads to human disease. ATRX is a member of the SWI2/SNF2 family of molecular motors which remodel chromatin by using the hydrolysis of ATP as an energy source. Mutations in ATRX give rise to a syndromal form of mental retardation associated with alpha thalassaemia (ATR-X syndrome). This represents one of the best studied examples of a newly recognised class of human disease, a disease of chromatin.

**Douglas Higgs** (FRS, DSc, FRCP, FRCPath FMedSci, member of EMBO) qualified in Medicine at King’s College Hospital Medical School (University of London) in 1974 and trained as a haematologist.  He joined the MRC Molecular Haematology Unit (Oxford) in 1977 and is currently Professor of Molecular Haematology at the University of Oxford, honorary consultant in the Department of Clinical Haematology (ORHA), Director of the MRC Molecular Haematology Unit (MHU) and Director of the Weatherall Institute of Molecular Medicine (WIMM).  The main interest of his laboratory is to understand how mammalian genes are switched on and off during differentiation and development using haematopoiesis as the experimental model. His laboratory has identified many of the principles underlying human genetic disease via their studies on thalassaemia. An important aspect of their current programme is to use genome editing to treat such diseases.

**Key Publications**

1. Gibbons RJ et al (2000) Mutations in the human SWI/SNF-like protein ATRX cause widespread changes in the pattern of DNA methylation. Nature Genet. 24, 368-371
2. Garrick D et al (2006) Loss of Atrx Affects Trophoblast Development and the Pattern of X-inactivation in Extra-embryonic Tissues PloS Genetics 2: e58
3. Law MJ et al (2010) ATR-X syndrome protein targets tandem repeats and influences allele-specific expression in a size dependent manner. Cell 143:367-78
4. Schenkel LC et al (2017) Identification of epigenetic signature associated with alpha thalassemia/mental retardation X-linked syndrome. Epigenetics and Chromatin 10:10

**Project 13**

**Project Title:** Role of lipid-specific T cells in human inflammation and translational development of new therapies to treat inflammatory disease

**Supervisor:** Professor Graham Ogg - [Graham.ogg@ndm.ox.ac.uk](mailto:Graham.ogg@ndm.ox.ac.uk)

**Project Overview**

Much of our understanding of human T cell immunology has been based on studies of peptide presentation by MHC molecules. It is becoming clear that non-peptide antigens can also be recognised by T cells, but presented by MHC-like molecules such as the CD1 family. CD1a is expressed at constitutively high levels by Langerhans cells of the skin where it is thought to present lipid antigens to T cells. We have recently made the observation that lipid-specific, CD1a-restricted T cells play a role in human skin inflammation, and have defined underlying mechanisms. However, the nature of the specific lipids presented by CD1a is still emerging, and so a major focus of the laboratory is on identifying new lipid antigens. We will use skin samples from our well-characterised cohorts of patients to define endogenous lipid antigen sources. The responding T cells will be characterised for T cell receptor repertoire and function; and mechanisms of antigen presentation will be investigated. We will collaborate with colleagues in Harvard University to identify the specific lipids through lipidomic studies, and if work progresses well, we will aim to solve the structural basis of CD1a lipid presentation through collaboration with colleagues in Monash University. Furthermore, we will optimise the lipids for agonist and antagonist activity for future therapeutic development.

Overall, the project will contribute to our understanding of fundamental molecular and cellular immunology applied to the study of human disease with direct translational implications.

**Training opportunities**

This translational project is based at the Weatherall Institute of Molecular Medicine, within the MRC Human Immunology Unit. There are excellent core facilities, infrastructure and expertise readily available. The student would receive training in molecular and cellular immunology techniques including cell culture, functional assays (ELISpot, intracellular FACS staining, bead array) and CD1a tetrameric complex staining. In addition the student would gain experience in handling human skin biopsies and growing T cells and antigen presenting cells, with concomitant assays including imaging, RNAseq, rtPCR and Western Blot. The student would also learn about regulatory issues surrounding the use and storage of human samples including ethics, hospital R&D, GCP and HTA. The student would attend GCP, statistical courses and relevant conferences and would also be able to attend the WIMM Techniques course which covers broad scientific techniques, as well as the excellent internal and guest speaker programmes available in the Weatherall Institute of Molecular Medicine.

**Supervisor profile**

Graham Ogg is investigating mechanisms underlying immunology with a focus on the skin. Specifically, his lab is studying the roles of lipid-specific T cells and innate lymphoid cells. Through collaborations with Harvard and Monash universities, his lab is identifying lipid antigens that contribute to disease pathogenesis and may be relevant to new therapeutic development. As a clinician, he sees patients with inflammatory skin disease and also translates the findings through to clinical trials.

**Key publications**

1. Stockenhuber K, Hegazy AN, West NR, Ilott NE, Stockenhuber A, Bullers SJ, Thornton EE, Arnold IC, Tucci A, Waldmann H, Ogg GS\*, Powrie F\* (\*joint senior authors). Foxp3(+) T reg cells control psoriasiform inflammation by restraining an IFN-I-driven CD8(+) T cell response. J Exp Med. 2018 Aug 6;215(8):1987-1998.
2. Hardman C, Chen Y-L, Salimi M, Jarrett R, Johnson D, Järvinen V, Owens RJ, Repapi E, Cousins D, Barlow JL, McKenzie A, Ogg G. CD1a presentation of endogenous antigens by group 2 innate lymphoid cells. Science Immunology 2017 pii: eaan5918.
3. Jarrett R, Salio M, Lloyd-Lavery A, Subramaniam S, Bourgeois E, Archer C, Cheung KL, Hardman C, Chandler D, Salimi M, Gutowska-Owsiak D, Bernardino de la Serna J, Fallon PG, Jolin H, Mckenzie A, Dziembowski A, Podobas EI, Bal W, Johnson D, Moody DB, Cerundolo V, Ogg G. Filaggrin inhibits generation of CD1a neolipid antigens by house dust mite-derived phospholipase. Sci Transl Med. 2016 Feb 10;8(325):325ra18
4. Cheung KL, Jarrett R, Subramaniam S, Salimi M, Gutowska-Owsiak D, Chen YL, Hardman C, Xue L, Cerundolo V, Ogg G. Psoriatic T cells recognize neolipid antigens generated by mast cell phospholipase delivered by exosomes and presented by CD1a. J Exp Med. 2016 Sep 26. pii: jem.20160258.
5. Bourgeois EA, Subramaniam S, Cheng TY, De Jong A, Layre E, Ly D, Salimi M, Legaspi A, Modlin RL, Salio M, Cerundolo V, Moody DB, Ogg G. Bee venom processes human skin lipids for presentation by CD1a. J Exp Med. 2015 Feb 9;212(2):149-63.

**Project 14**

**Project Title:** Exploring the basis of cardiac remodelling in hypertrophic cardiomyopathy: the role of the immune system

**Supervisor:** Professor Hugh Watkins - [hugh.watkins@rdm.ox.ac.uk](mailto:hugh.watkins@rdm.ox.ac.uk)

**Project Overview**

Hypertrophic cardiomyopathy is the most common inherited cardiac disorder, and was the first to be understood at a molecular genetic level. We, and others, have shown that HCM is caused by diverse heterozygous mutations in genes encoding components of the contractile apparatus of heart muscle. The primary biophysical consequences of the myofilament mutations are increasingly understood, as are the final common pathways of cardiomyocyte dysfunction. This has led to ongoing trials of novel treatments. However, major secondary changes, most notably fibrosis, occur in the non-myocyte compartment that will limit treatment efficacy in established disease.

We have shown that there is metabolic crosstalk between stressed cardiomyocytes and neighbouring cells, and our preliminary data indicate that this, and other aspects of remodelling, are mediated, at least in part, by cells of the immune system that accumulate in HCM myocardium. We have shown that ablation of the adaptive immune system markedly worsens remodelling in a well-validated HCM mouse model. We hypothesise that local cardiac immune activity, both acquired and innate, plays an essential and dynamic role in HCM with, as is typical in immunity, a balance of deleterious and protective effects. We will test this by identifying the role of specific immune components in the progression of HCM using reductionist experiments in HCM mouse models, studies in affected human myocardium and large-scale human genetic interrogation. Identification of the immune activity involved will inform novel disease modifying therapy for established disease.

**Training opportunities**

Depending on the prior experience of the successful candidate, this project would provide training in creation and analysis of mouse models, cardiac phenotyping of mouse models, molecular and cellular tools for investigating immune cell subsets. The project will employ cutting edge single cell genomic approaches, well-established *in vivo* mouse models, advanced immunological and imaging techniques (e.g. FACSymphony, cell sorting, CyTOF, two-photon confocal fluorescence microscopy, and light sheet microscopy). Hands on supervision will be provided by an experienced post-doctoral scientist/immunologist working on this project, Dr Ying-Jie Wang.

**Supervisor**

Professor Hugh Watkins is the Head of the Radcliffe Department of Medicine and a group leader in the Wellcome Centre for Human Genetics. He is a clinician scientist who uses molecular genetic analysis of cardiovascular disease as a tool to define disease mechanisms and therapeutic targets. He is best known for his work on inherited heart muscle diseases, in particular hypertrophic cardiomyopathy. His work on genetic causes of this, and other, ‘sudden cardiac death’ syndromes has been translated into clinical practice, with adoption in international clinical guidelines and commissioning of a national DNA diagnostic service for the NHS. Clinical trials are underway of new disease modifying therapies targeted to the energy deficiency that his group has identified as a key component of cardiomyopathy pathogenesis. He is a Fellow of the Academy of Medical Sciences and a Fellow of the Royal Society.

**Key publications**

1. Watkins H, Ashrafian, Redwood C. Mechanisms of Disease: Inherited Cardiomyopathies. *New Engl J Med* 2011; 364:1643-56.
2. Yavari, A., Bellahcene, M., Bucchi, A., Sirenko, S., Pinter, K., Herring, N., . . . Watkins H, Ashrafian, H. (2017). Mammalian γ2 AMPK regulates intrinsic heart rate. *Nature Communications*, *8*(1), 1258. (H. Watkins joint senior author).
3. Robinson P, Liu X, Sparrow A, Patel S, Zhang YH, Casadei B, Watkins H, Redwood C. Hypertrophic cardiomyopathy mutations increase myofilament Ca2+ buffering, alter intracellular Ca2+ handling, and stimulate Ca2+-dependent signaling. *J Biol Chem*. 2018;293:10487-10499.
4. Toepfer CN, Wakimoto H, Garfinkel AC, McDonough B, Liao D, Jiang J, Tai AC, Gorham JM, Lunde IG, Lun M, Lynch TL 4th, McNamara JW, Sadayappan S, Redwood CS, Watkins HC, Seidman JG, Seidman CE. Hypertrophic cardiomyopathy mutations in MYBPC3 dysregulate myosin. *Sci Transl Med*. 2019 Jan 23;11(476).
5. Ariga R, Tunnicliffe EM, Manohar SG, Mahmod M, Raman B, Piechnik SK, Francis JM, Robson MD, Neubauer S, Watkins H. Identification of Myocardial Disarray in Patients With Hypertrophic Cardiomyopathy and Ventricular Arrhythmias. *J Am Coll Cardiol*. 2019 May 28;73(20):2493-2502.

**Project 15**

**Project Title:** Modelling of non-typeable *haemophilus* influenzae / rhinovirus interactions in lower airways inflammation in mouse and human

**Supervisor:** Dr Timothy SC Hinks - [timothy.hinks@ndm.ox.ac.uk](mailto:timothy.hinks@ndm.ox.ac.uk)

**Project overview**

Aim: to discover the immunological mechanism underlying the dramatic efficacy of macrolide molecules in reducing exacerbations in asthma. Our emerging data suggest this effect is related to non-typeable*haemophilus influenzae* (NTHi) infection, likely via an interaction with rhinovirus (RV).

Asthma affects 350 million people worldwide. NTHi is a major cause of mucosal infections such as exacerbations of chronic obstructive pulmonary disease and asthma, otitis media, sinusitis and also invasive disease including pneumonia and meningitis. Little is understood about how this pathogen establishes a chronic infection on mucosal surfaces which can prove very resistant to antibiotic treatment and can drive inflammation which does not respond to treatment with inhaled steroids. We believe NTHi establishes a persistent infection in neutrophilic asthma and predisposes to enhanced susceptibility to rhinovirus-induced exacerbations.

In collaboration with Harwell MRC we are developing unique murine models using mice genetically susceptible to *haemophilus* infections. We also use human primary bronchial epithelial cells from our bronchoscopy programme and grown at air liquid interface. In this project the DPhil candidate will use cutting edge techniques including high-parameter flow-cytometry and CITE-seq and chip-cytometry to exploit both these models. They will investigate how NTHi drives steroid-resistant inflammation in allergic airways disease and how NTHi interacts with RV *in vitro* and *in vivo*.

**Training opportunities**

The DPhil candidate will acquire expertise in cellular immunology, using murine models of human disease, in vitro human air-liquid interface culture, cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq), multiparameter flow-cytometry, chip cytometry, immunofluorescent fluorescent imaging and microbiological techniques.

**Supervisor**

Dr Timothy SC Hinks is a Wellcome Trust Career Development Fellow leading a group researching the immunology of airways diseases. As an NHS Consultant in Respiratory Medicine he co-leads the Oxford Specialist Airways service with Ian Pavord, providing secondary and tertiary care for people with unstable, difficult and severe asthma at the John Radcliffe Hospital in Oxford. Tim studied medicine at Cambridge and Oxford. He then researched novel T cell-based immunodiagnostics for tuberculosis as a research fellow in Oxford. In 2007 he moved to Wessex as an Academic Clinical Fellow to undertake higher clinical training in respiratory medicine. Funded by a Wellcome Training Fellowship his doctoral research in Southampton with Ratko Djukanovic and Stephan Gadola was into the roles of innate and adaptive T cells in the human airways in asthma. On a Wellcome Postdoctoral Clinical Fellowship with James McCluskey at the Peter Doherty Institute, University of Melbourne he researched the basic biology of mucosal associated invariant T cells in bacterial and viral respiratory infections and in tissue repair.

The current focus of his research group, funded by a Wellcome Career Development Fellowship and the prestigious Beit Fellowship, is defining the innate-like mucosal T cell response to bacterial infection in airways disease, specifically *Haemophilus influenzae* and related organisms in airways inflammation in severe asthma, and the immune mechanisms underlying macrolide efficacy in asthma. His group are also investigating the genetics, transcriptomics and epigenetics of severe asthma in a large cohort and using bronchoscopies.

**Key publications**

1. 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 [*In press*] <https://doi.org/10.1101/490649> Available on bioRxiv <https://www.biorxiv.org/content/early/2018/12/09/490649>

1. van Wilgenburg B, Loh L, ChenZ, Pediongco TJ, Wang H, Shi M, Zhao Z, Koutsakos M, NüssingS, 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

1. Wang H, D’Souza C, Lim XY, Kostenko L, Pediongco TJ, Eckle SBG, Meehan BS, Wang N, Li S, Liu L, Mak JYW, Fairlie DP, Iwakura Y, Gunnersen JM, Stent AW, Rossjohn J, Westall GP, Kjer-Nielsen L, Strugnell RA, McCluskey J, Corbett AJ, **Hinks** **TS**, Chen Z

*Nat Commun*. 2018 Aug 22;9(1):3350. doi: 10.1038/s41467-018-05202-8

1. **Hinks TS**, Wallington JC, Williams AP, Djukanovic R, Staples KJ, Wilkinson TM. Steroid-induced deficiency of mucosal-associated invariant T cells in the COPD lung: implications for NTHi infection.

*Am J Respir Crit Care Med* 2016 Nov 15;194(10):1208-1218

1. Hilvering B, **Hinks TSC,** Stöger L, Marchi E, Salimi M, Shrimanker R, Liu W, Go S, Powell T, Chen W, Luo J, Thulborn S, Kurioka A, Leng T, Mattehws J, Connolly C, Borg C, Willberg CB, Ramasamy A, Djukanovic D, Ogg G, Pavord I, Klenerman P, Xue L. Synergistic activation of pro-inflammatory type-2 CD8+ T lymphocytes by lipid mediators in severe eosinophilic asthma.

*Mucosal Immunol.* 2018 Jun 15. doi: 10.1038/s41385-018-0049-9 [**Joint first author**]

**Project 16**

**Project Title:** Personalised immunotherapy treatment to manage malignant pleural effusion progression (the TAILOR study)

**Supervisor:** Professor Najib Rahman - [najib.rahman@ndm.ox.ac.uk](mailto:najib.rahman@ndm.ox.ac.uk)

**Background**

Malignant pleural effusion (MPE) is the accumulation of fluid in the pleural cavity (the area between the lungs and the chest wall) due to cancer and is a marker of advanced malignancy. MPE is frequent, affecting approximately 15% of cancer patients (500-700 of million population every year) and independently of the primary cancer signals shortened life expectancy (3-12 months) and severely compromised quality of life due to breathlessness and chest pain. MPE remains refractory to current treatments and management is mainly symptomatic without specific control of cancer progression and fluid accumulation.

T cells are a subtype of lymphocytes which are responsible for the adaptive immunity and play a key role for the immune surveillance and tumour suppression. Cytotoxic CD8+ T cells are the preferential candidate for T cell-based immunotherapy. However, due to the tumour microenvironment, T cells display heterogeneous and disturbed phenotypes such as exhaustion which is often characterised by loss of functions and transcriptomic alterations. It is known that cancer specific T cells exist in MPE. However, it remains to be answered whether these T cells can be employed in immunotherapy to control the progression of the cancer.

Tumour heterogeneity among patients emphasises the need to redesign clinical stratification algorithms with a view to manage patients with the most appropriate treatment. T cells are a potent anti-cancer weapon that can be useful for the development of immunotherapy based personalised treatments.

**Project overview**

We have developed a methodology to establish patient derived cancer (PDC) cell lines from MPE specimens. These PDC cell lines truly represent the tumour and can be used as an *ex vivo* platform to develop and assess the efficiency of anticancer therapies. The PDC cells show different response profiles to anticancer agents mirroring the interpersonal tumour variability. Furthermore we have valuable experience in studying cancer specific T cells from various types of malignancies and have developed techniques to characterise T cell phenotype and function. The thorough investigation and cross examination of PDC cell lines and paired matched cancer specific T cells (derived from the same patient), would be a unique opportunity to investigate and assess the T cell immunotherapy to arrest tumour growth.

The aim of the study is to a) characterize the distinct phenotype and function of cancer specific T cells in MPE, b) to understand the interaction between T cells and tumour microenvironment and c) ultimately develop and assess the efficiency of the T cell based immunotherapy for MPE patients.

**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.

**Supervisors**

Professor Najib Rahman runs the Oxford Pleural Unit, Directs the Oxford Respiratory Trials Unit and conducts research in pleural disease at the Oxford Centre for Respiratory Medicine. He was appointed Director of the Oxford Respiratory Trials Unit, Consultant and Lead for Pleural Disease in Oxford in 2011. His main focus of research is the design and delivery of practice changing multicentre clinical trials in pleural disease, and is currently involved in randomized and observational studies in pleural infection, pneumothorax and malignant pleural effusion intervention. He is trained in Thoracoscopy, Thoracic Ultrasound and Clinical Trials methodology, and has published over 150 papers with citations of >6000 in the fields of pleural disease and thoracic ultrasound.

Professor Tao Dong is the director of the Chinese Academy of Medical Sciences Oxford Institute. Moreover, Tao is the principal investigator of the Centre of Translational Research, a centre of excellence in immunology. The main objective of her research is focused on the functional aspects of the antigen specific T cells and study the factors affecting T cells in controlling virus infection and cancer development. Tao is a high-profile leader in immunology research and her work has been published in high impact factor journals.

Dr. Nikolaos Kanellakis is the leading research fellow of the Laboratory of Pleural Translational Research. His research has been focused into pleural diseases and malignancies, and he has been involved in laboratory translational studies and clinical trials. His interests include the development of *ex vivo* models suitable to investigate diseases and develop therapies.

**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. *JAMA*. 2012

**Project 16**

**Project Title:** Identification of key determinants affecting the quality of human Cancer specific cytotoxic T cells

**Supervisor:** Professor Tao Dong – [tao.dong@imm.ox.ac.uk](mailto:tao.dong@imm.ox.ac.uk)

**Project Overview**

Cancer individuals are characterized by dysfunctional anti-tumor T-cell responses. In recent years, multiple inhibitory receptors, known as immune checkpoint receptors, expressed on T cells, have been identified. Recent exiting results from clinical trials with antibodies targeting these inhibitory receptors, such as PD-1 and CTLA4, have demonstrated their ability to activate anti-tumour immunity in a broad range of cancer patients, with dramatic therapeutic results. However only a small proportion of the patients responded to currently available immune-checkpoint blockade therapy. One of the main aims of our group is to explore the potential targets for immunotherapy which could improve the efficacy of current immunotherapeutic strategies, several *ex vivo* and *in vitro* T cell platforms with most advanced technologies (such as CyTOF, single cell RNASeq with 10X Genomics and SmartSeq 2) have been set up and optimised in our group. These cutting edge platforms enabled us to characterise Cancer-specific T cells isolated from cancer patients, to understand the correlation between “dysfunctional” T cell responses and ineffective control of cancer cells, and to identify key determinants affecting the quality of human Cancer-specific cytotoxic T cells.

We have performed single cell analysis with specific subsets of T cells isolated from cancer tissues and have identified unique molecular signatures in clonal expanded cell subsets. This D.Phil project will be focused on evaluating the potential of these newly identified molecules as target for future immunotherapy.

**Training Opportunities**

The student will receive formal training in flow cytometry, cell culture, T cell cloning, full range of assays to evaluate T cell function , T cell receptor repertoire analysis , CyTOF, RNASeq and bioinformatics.

**Supervisors**

The student will be mentored by Professor Tao Dong and Dr. Yanchun Peng.

Professor Tao Dong is the director of the Chinese Academy of Medical Sciences Oxford Institute. Moreover, Tao is the principal investigator of the Centre of Translational Research, a centre of excellence in immunology. The main objective of her research is focused on the functional aspects of the antigen specific T cells and study the factors affecting T cells in controlling virus infection and cancer development. Tao is a high-profile leader in immunology research and her work has been published in high impact factor journals.

**Relevant publications (All were D.Phil projects)**

1. ABD HAMID M Et al , Efficient anti-tumor effector responses by metabolically energetic CD103+ T cells with accelerated T cell apoptosis, under revision for Cancer Immunology Research. (Ms available upon request)
2. Li, x et al, A comprehensive analysis of key immune checkpoint receptors on tumor infiltrating T cells with implication of anti-cancer immune therapy revision submitted to Frontiers Oncology. (Ms available upon request)
3. ABD HAMID M Et al, 2019. Enriched HLA-E and CD94/NKG2A interaction limits antitumor CD8+ tumor-infiltrating T lymphocyte responses. *Cancer Immunol Res*, 7(8), pp. 1293-1306.
4. Hang C, et al 2018 Et al, Genetic abrogation of immune checkpoints in antigen-specific cytotoxic T-lymphocyte as a potential alternative to blockade immunotherapy. *Sci Rep*, 8 (1), pp. 5549.
5. LEE LY-H et al, Memory T cells established by seasonal human influenza A infection cross-react with avian influenza A (H5N1) in healthy individuals *2008, J Clin Invest*, 118 (10), pp. 3478-3490.

**Project 17**

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

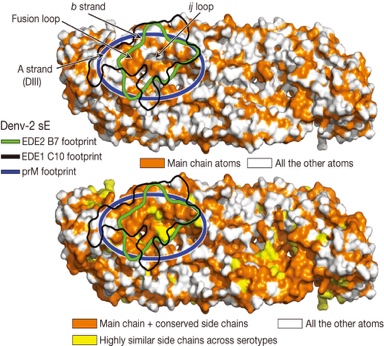
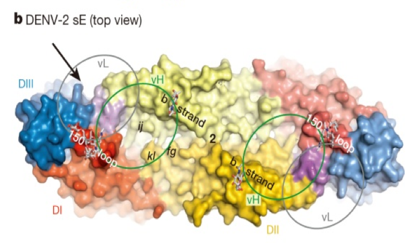
**Supervisor:** Professor Gavin Screaton - [gavin.screaton@medsci.ox.ac.uk](mailto:gavin.screaton@medsci.ox.ac.uk)

**Project Overview**

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

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

Top view



C



Side View

A

B

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

**Training opportunities**

The student will join a team having more than 20 years experience in virology, immunology and molecular biology. Our work has made a great contribution to the field with a number of high impact publications. The student will be trained by experienced post-docs in a broad range of techniques such as basic virology (viral propagation, neutralisation and viral titration), immunology (ELISA, Immuno-precipitation, SDS-PAGE, Western blot, FPLC and affinity purification, Flow cytometry, Single cell sorting, tissue/cell culture, molecular biology (PCR, using software programs to design primers, mutagenesis, deep sequencing of antibody repertoire, cloning, protein expression in bacteria, yeast, insect and mammalian cells systems) generating monoclonal antibodies from single human and mouse B cell, using software and structure analysis to design new immunogens, and mouse handling.

**Supervisor:** Professor Gavin Screaton

**Co-Supervisor:** Dr. Juthathip Mongkolsapaya

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

**Key publications**

1. Wilder-Smith, A. et al. Deliberations of the Strategic Advisory Group of Experts on Immunization on the use of CYD-TDV dengue vaccine. Lancet Infect Dis, doi:10.1016/S1473-3099(18)30494-8 (2018).
2. Screaton, G., Mongkolsapaya, J., Yacoub, S. & Roberts, C. New insights into the immunopathology and control of dengue virus infection. Nat Rev Immunol 15, 745-759, doi:10.1038/nri3916 (2015)
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**Project 18**

**Project Title:** Study of broadly protective human monoclonal antibodies to influenza neuraminidases

**Supervisor:** Professor Alain Townsend - [alain.townsend@imm.ox.ac.uk](mailto:alain.townsend@imm.ox.ac.uk)

**Project Overview**

Neuraminidase (NA) is one of the surface glycoproteins of influenza virus. There are 11 subtypes of neuraminidase and only N1 and N2 are in seasonal human influenza viruses. Other neuraminidases are seen in avian influenza viruses.

Monoclonal antibodies to neuraminidase are highly protective, but they have not been explored in as much detail as antibodies to haemagglutinin. Our lab has been characterizing human monoclonal antibodies to neuraminidases in collaborations with Dr Kuan-Ying Huang ((Division of Paediatric Infectious Diseases, Chang Gung Children’s Hospital, Taiwan) and Dr Bei Bei Wang (Beijing Ditan Hospital, China) (Rijal et al 2019, bioxRIV). One of the antibodies isolated from a H7N9 convalescent child in China was found to broadly cross-inhibit the NA activity of N1, N2, N9 and N6 neuraminidases (so far tested). The structural analysis done by Prof George Gao’s group (Chinese Academy of Medical Sciences) revealed that it inserts a long CDR3 loop into the conserved catalytic site of the enzyme (Ziang et al unpublished). The core enzymatic site is conserved in all the neuraminidases.

This suggested that it is possible to isolate broadly reactive neuraminidase antibodies that target the catalytic site of neuraminidase, although the antibody we studied was limited due to the contacts to the peripheral non-conserved residues. The knowledge obtained from studying such antibodies will help in improvement of vaccine design and the development of therapeutic antibodies, bicyclic peptides or small molecules.

The DPhil project has following outline and aims:

*1) I*solation and characterisation of more antibodies to avian neuraminidases

We will isolate more monoclonal antibodies and characterise them for their binding and inhibition properties.

*2) Exploring therapeutic potential of the selected antibodies*

From the numbers of isolated antibodies, we will test the most potent ones for protection of animal models against the lethal dose of virus infection.

*3) Analysing the structure of the antibodies in complex with their antigens*

Once we have a selection of potent and protective antibodies, we will study the binding epitopes that might reveal the protective features/mechanism of protection by the antibodies.

*4) Looking into ways to improve the function of antibodies*

Based on the structural analysis, we can try to improve the function of antibodies via site directed mutagenesis. This approach has already shown promise in broadening the reactivity of an antibody to N9 neuraminidase by optimising a salt bridge identified in crystallographic analysis in collaboration with George Gao’s group.

**Reference**

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**Project 19**

**Project Title:** Molecular basis of moonlighting enzymes – the Swiss army knives in a cell

**Supervisor:** Professor Wyatt Yue – [wyatt.yue@sgc.ox.ac.uk](mailto:wyatt.yue@sgc.ox.ac.uk)

**Project Overview**

While metabolic enzymes classically catalyse a chemical reaction within a pathway, emerging data point to certain metabolic enzymes that moonlight in non-metabolic, and even non-enzymatic functions. These additional functions are often mediated by protein-protein and protein-RNA interactions, within the context of macromolecular complexes. Characterization of these interaction networks are key to deciphering how moonlighting enzymes multi-task, and how they impact on the phenotypic spectrum of human diseases.

**Training Opportunities**

This DPhil project will combine structural, biochemical, and proteomic approaches, to chart the mechanistic landscape of enzyme moonlighting using two different examples, one involving a short-chain dehydrogenase in tRNA processing, and the other an amino-acyl tRNA synthetase in regulation of cell growth.

*Through this project the student will:*

* map out the interaction network of these moonlighting enzymes using proteomics approaches
* gain a solid grounding in cutting edge cryo-electron microscopy and crystallography to elucidate complex architecture and assembly
* develop bespoke biophysical and cellular assays to probe the moonlighting functions, through engagement with our cell biology and clinical collaborators
* apply an *in vitro* selection platform for the generation of antibody fragments, as cellular probes for functional studies and therapeutic development of these enzymes

**Supervisor:** Wyatt Yue, SGC Oxford, Nuffield Department of Medicine

**Project 20**

**Project Title:** How is alpha thalassaemia acquired in the context of myelodysplasia

**Supervisor:** Professor Doug Higgs – [doug.higgs@imm.ox.ac.uk](mailto:doug.higgs@imm.ox.ac.uk)

**Project Overview**

Thalassaemia is the most common form of inherited anaemia throughout the world. In all cases, it results from an imbalance in the production of the -like and -like globin chains of haemoglobin, leading to -thalassaemia and -thalassaemia respectively. The aim of our laboratory is to understand how the globin gene clusters are normally regulated during development and differentiation and how this is perturbed in patients with thalassaemia. By approaching these questions, we are also developing a general understanding of how mammalian genes are normally switched on and off during erythropoiesis and identifying many general principles underlying human molecular genetics.

During the course of this work, we have identified about 130 patients who have a rare form of -thalassaemia which occurs in the context of a pre-malignant condition called the myelodysplastic syndrome (MDS). These patients have no pre-existing forms of -thalassaemia (AT) and so this condition is acquired specifically in the pre-malignant clones of cells in MDS: hence the condition is referred to as the ATMDS syndrome. When we analyse the bone marrow cells of patients with ATMDS we find a distinct constellation of mutations which are also found in other patients with MDS but, importantly, in addition, most patients with ATMDS have mutations in a chromatin remodelling factor called *ATRX*. This protein was discovered in our laboratory in 1995 as a cause of X-linked -thalassaemia associated with developmental abnormalities (ATR-X syndrome) and *ATRX* has more recently been recognised as a tumour suppressor gene in a variety of malignant tumours. Importantly, the severity of -thalassaemia in ATMDS syndrome is much greater than that seen in ATR-X syndrome even when the ATRX mutations involved are similar or the same.

The key question in this project is how do mutations in ATRX down regulate -globin gene expression in ATMDS syndrome and why is the effect so much greater in ATMDS compared with ATR-X syndrome. We have been studying both primary cells from patients with ATMDS or ATR-X syndrome and developing much needed erythroid cell models of these diseases. This has been challenging for a variety of reasons but recently we have shown that using single cell analysis we are now able to identify a sub-population of erythroid cells that appear to be more affected by ATRX mutations than others and we are currently investigating why this should be so. Some clues to this will come from analysing the impact of other genes that are mutated in ATMDS syndrome: appropriate cell lines in which such genes have been mutated individually and in combination are now edited and available. The aims of this project will therefore be to further characterise primary cells and the recently established erythroid cell models of ATMDS syndrome using transcriptional, epigenetic and chromosome conformation studies to analyse how -globin expression is perturbed in this severe, acquired form of -thalassaemia. All of such experimental approaches are well established in our laboratory. This project will contribute to our understanding of globin gene regulation, the mechanism(s) by which chromatin remodelling factors normally work and how they may contribute to malignant diseases when mutated.

**Training Opportunities** (both Laboratory and clinical)

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

**Supervisors:**

Primary: Professor Doug Higgs FRS

Clinical (if Primary supervisor is not clinician):

CAMS co-supervisor (optional): Professor Richard Gibbons DPhil FRCP

**Supervisor’s short profile and links to web profile**

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

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

**Key publication list links to proposed project**

1. Gibbons RJ, Picketts DJ, Villard L & **Higgs DR** (1995) Mutations in a putative global transcriptional regulator cause X-linked mental retardation with -thalassemia (ATR-X Syndrome). *Cell*, **80**, 837-845. (IF: 31, citations 540)
2. Gibbons RJ, Pellagatti A, Garrick D, Wood WG, Malik N, Ayyub H, Langford C, Boultwood J, Wainscoat JS & **Higgs DR** (2003) Identification of acquired somatic mutations in the gene encoding chromatin-remodelling factor ATRX in the α thalassaemia myelodysplasia syndrome (ATMDS). *Nat Genet*, **34**, 1-4. (IF: 27, citations 135)
3. Steensma DP, Gibbons RJ & **Higgs DR** (2005) Acquired -Thalassemia in Association with Myelodysplastic Syndrome and Other Hematologic Malignancies. *Blood,* **105,** 443-452. (IF: 13, citations 105)