CHINESE ACADEMY of MEDICAL SCIENCES OXFORD INSTITUTE

NUFFIELD DEPARTMENT of MEDICINE

UNIVERSITY OF OXFORD

CSC-COI SCHOLARSHIP DPHIL PROJECT PROPOSALS

Enrolment Year: 2025

41 Projects Available

- ☑ MSD Program: 1 Program
- ☑ Cancer Biology & Immunology: 7 Projects
- ☑ Infection, Inflammation & Disease: 12 Projects
- ☑ Vaccine and Antibodies: 5 Projects
- ☑ T Cell Immunology: 4 Projects
- ☑ Structure Biology: 4 Projects
- ☑ Other Themes: 8 Projects

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1. MSD Programs

1.1 Genomic Medicine and Statistics DPhil Programme

Prof. Julian Knight (Course Lead)

Contact Email Address:

well-gms-administrators@well.ox.ac.uk

Programme Overview

There is a unique opportunity for students to be trained in the rapidly growing area of genomic medicine, and the related areas of bioinformatics, big data science and statistics. Scholarship funding is available to support two students from China each year to join the highly prestigious DPhil programme in Genomic Medicine and Statistics at the University of Oxford.

This is a four-year DPhil programme aiming to train future scientific leaders who will work at the cutting edge of genomics in biomedical research and the clinic. Students from a diverse range of academic backgrounds are encouraged to apply, from analytical sciences (including maths, physics, computer science) to wet lab biomedical sciences (including molecular biology, human genetics, biology, and medicine).

The course is part of the University of Oxford Medical Sciences Division Doctoral Training Centre and hosted by the Centre for Human Genetics.

The first year includes taught modules focused within the first term. First-year students then undertake short research projects in up to three laboratories in three-month rotations, with further training and teaching sessions tailored to the needs of individual students. The research projects and lab visits help inform the choice of DPhil project to be undertaken over the subsequent three years of the programme. The final three years of the course will comprise a doctoral research project under the supervision of two named supervisors and a doctoral committee. Students do not need to choose a research project in advance of applying to the course, this decision is made at the end of their first year after the rotations.

The programme is focused on genomic and -omic technologies, functional genomics, genome biology, genomics of disease, genomic analysis, drug discovery and application of genomics in the clinic. The names of principal investigators currently affiliated with the programme are shown here together with current research projects.

For further details see https://www.ox.ac.uk/admissions/graduate/courses/dphil-genomic-medicine-and-statistics

Student candidates/selection criteria

Eligible students

Chinese students eligible for the program include those enrolled on an eight-year MD course (20 Scholarships) or undergraduates of non-medical courses (5 Scholarships) from 14 China's top Medical Universities. Scholars must demonstrate exceptional academic merit and/or potential prior to commencing a full-time DPhil course of study in any subject within the Medical Sciences Division of the University. Scholars shall meet the standard selection criteria of the University and the specific course requirements here.

Shortlist and Interview

CSC and COI will be responsible for sending out the advertisement and checking students against their own eligibility criteria for shortlisting. The University will shortlist and interview candidates of the Programme using the University's own entry requirements for this course. Successful candidates will have passed both the University and CSC application process.

Course coordinators

Oxford: Professor Julian Knight

CSC: Xiaopeng Hu

Application and selection process

Only candidates who are recommended by three Professors in China and subsequently offered admission to study at the University of Oxford, having applied through the normal admissions process, and meet the criteria for funding by CSC, can be considered for scholarships of the Programme. Applying students will select the Genomic Medicine and Statistics course option on the form and then be assessed as part of a gathered field with other students applying to the Genomic Medicine and Statistics course who have not applied for this scholarship scheme. The selection of candidates for the scholarships will be the joint responsibility of the CSC and the University. Final selection will be based solely on academic merit and/or potential.

Graduate destinations

The interdisciplinary nature of the programme is reflected in the destination of graduates which includes academic research in prestigious laboratories worldwide together with biotech, spin-outs, consulting and working in health care settings.

Key dates (tentative and subject to change)

15th Oct 2024	CSC/COI/NDM begin advertising for applications.
20th Nov 2024	Deadline for candidates to submit to CSC their CV, cover letter and indicate they are applying for the Genomic Medicine and Statistics course.
1st Dec 2024	Deadline for candidates to apply directly to Oxford University for admission.
Early Jan 2025	Oxford University will shortlist candidates for the Genomic Medicine and Statistics course.
Late Jan 2025	Interviews by Oxford University for the Genomic Medicine and Statistics course.
Early Feb 2025	Interviews by CSC.
Mar 2025	Successful candidates will be notified.

Disease Relevance

The Genomic Medicine and Statistics course is designed to provide world class training and the opportunity to conduct research in the rapidly developing field of genomic science and its application to human health. The research opportunities for the doctoral research are very broad and interdisciplinary, with collaboration highly encouraged. Disease areas span the breadth of clinical medicine, from infection and immunity to cardiovascular disease, metabolism, rare disease and cancer across Departments in the Medical Sciences Division.

Key Technology

The programme is focused on the following themes:

- Genomic and -omic technologies (including method development, single cell genomics, imaging, model systems, CRISPR screens, genome engineering, proteomics, metabolomics, high throughput screening)
- Functional genomics (gene regulation and epigenetics)
- Genome biology (genetic variation, recombination, human history, evolution, palaeogenomics, pathogen genomes)
- Genomics of disease (Mendelian, multifactorial traits, cancer)
- Genomic analysis (bioinformatics and statistical genetics)
- From genes to clinical proof of concept (integrated drug development pipeline spanning genetic-led target discovery, structural biology, medicinal chemistry)
- Application of genomics in the clinic (rare disease diagnostics, cancer therapeutics, personalised medicine and genome therapies).

Teaching modules combine theoretical and practical classes, with further skills training available through the Medical Sciences Doctoral Training Centre.

Training Opportunities

GMS Teaching Overview

The GMS DPhil programme includes a first term of teaching and up to three lab rotations before deciding on the final DPhil project. Students offered a place on the programme come from diverse academic disciplines and the teaching gives a broad introduction to a variety of genomic and statistical topics. The research projects and lab visits help inform the choice of DPhil project to be undertaken over the subsequent three years of the programme.

The aims of the first term are to:

- provide cross-discipline skills training in genome biology, statistics and bioinformatics
- introduce the latest genomic techniques and applications
- emphasise the tight integration of lab and computational work in genomic research
- discuss research activity in the wider context of clinical genomic medicine
- familiarise students with research areas, potential supervisors and projects

The final three years of the course will comprise doctoral research under the supervision of two named supervisors and a doctoral committee. Applicants are advised to visit the Doctoral Training Centre course webpage for further information about supervisors connected to this programme.

Students receive world-class training, supervision, mentorship and pastoral support. Promoting excellence in research culture underlies all aspects of the programme with a commitment to support creativity, prioritise diversity and inclusion, and promote best practice.

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2. Cancer Biology & Immunology

2.1 Epithelial-mesenchymal interdependence in intestinal regeneration and neoplasia

Prof. Simon Leedham & Prof. Bethan Psaila

Contact Email Address:

Simon.leedham@well.ox.ac.uk;

bethan.psaila@ndcls.ox.ac.uk

Project Overview

In the intestinal mucosa the epithelial stem-to-differentiation fate spectrum along the crypt-villus axis is mirrored by reciprocal functional segregation of underlying stromal cells, and both cell compartments are regulated by the establishment of opposing gradients of morphogenetic signalling (particularly Wnt and BMP signalling). The interaction and co-evolution of these embryologically distinct cellular compartments strictly regulates homeostatic epithelial cell fate, with appropriate stem cell activity and cell proliferation restricted to the base of the crypt in health. However, the complexity of this epithelial-mesenchymal interdependence enables the tissue to be exquisitely sensitive and responsive to local change, with adaptive reprogramming inducing stem cell behaviour which is required for a rapid physiological response to external perturbations [1]. This enables rapid functional adaptations to dietary, infectious or chemical alterations and enforces regeneration of injured epithelium. Unfortunately, it also renders the mucosa susceptible to pathological dysregulation, especially following mutation-induced disruption of the key regulatory pathways in the initiation of neoplasia [2]

This project will use mouse models and human tissue to explore this intercompartmental interdependence, looking to examine how epithelial and mesenchymal cell compartments phenotypically and epigenetically change in response to tissue injury and neoplasia-initiating mutations.

Sub-project 1. Spatio-temporal mapping of the intercompartmental response to intestinal ulceration.

Here we will use an endoscopic biopsy model of mouse colonic wounding to induce acute tissue injury and spatio-temporally explore the cross-compartmental wounding response over a repair process that takes about 6 days from ulceration to restitution. We will combine spatial biology techniques (such as CosMx/Xenium spatial transcriptomics) alongside sequencing technologies (RNA and ATAC Seq) to explore the chromatin landscape underpinning responsive mesenchymal cell transcriptional/phenotypic reprogramming following acute epithelial cell loss. In particular, we will explore the cellular constituents and organisation of regenerative stem cell niches that enable residual epithelial stem cell plasticity during the repair process [3]. Finally, we will contrast mouse findings with endoscopic biopsy tissue from patients with Ulcerative colitis and Crohn's disease to see if murine findings are replicated in human inflammation and ulceration disease settings.

Sub-project 2. Assessing the impact of successful initiating mutations on epithelial-mesenchymal cell interactions.

In the intestine, multiple mutations can be identified in apparently normal tissue [4], hence not all acquired mutation initiates neoplasia. There is likely to be a degree of homeostatic buffering from the tissue microenvironment that must be overcome for an epithelial mutation

to induce tumourigenesis. Successful initiating mutations (such as those in the Wnt and BMP pathway) not only bestow an epithelial cell-intrinsic fitness advantage, they can also simultaneously remodel the tissue microenvironment through disruption of signalling interaction with surrounding mesenchymal cells[5] and induced non genetic immune evasion. We will explore this impact by inducing timed initiating mutation in different cell types in mouse models and then using temporally-spaced sequencing and spatial biology analysis[6] to assess how epithelial mutation alters key cell interactions in surrounding stromal and immune cell populations. We will contrast findings in mouse models with the analysis of different subtypes of early human polyps resected endoscopically.

Disease Relevance

The physiological processes that underpin intestinal regeneration including mesenchymal cell activation, epithelial cell reprogramming/cell plasticity and immune cell infiltration are reliant on a co-evolving and interdependent relationship between the epithelium and the surrounding mesenchyme. These processes are co-opted and corrupted in neoplasia initiation and propagation. However, the majority of therapies for inflammatory bowel disease focus on reducing inflammation to allow healing by secondary intention, rather than by enhancing tissue restitutive capabilities. In a similar vein, most cancer drugs are targeted solely at the proliferating cancer epithelium, without looking to manipulate the bidirectional interaction between cell compartments. Through understanding the interdependent relationship between the epithelial and mesenchymal cell compartments in a regenerative and neoplastic setting, we hope to identify potential new targets for novel therapies, as well as understanding evolved mechanisms of existing drug tolerance and acquired resistance.

Key Technology

- Spatial biology – Oxford has access to multiple spatial biology platforms including single cell spatial transcriptomics (CosMx and Xenium) and multiplex IHC.
- Multiscale spatial analysis – together with colleagues in Oxford Mathemetics (Josh Bull, Helen Byrne) we have been working towards the development of a multiscale spatial analysis platform (MuSpAn). This will enable advanced analysis of any generated spatial biology image, allowing quantative assessment of cross-compartmental cell interactions across a range of tissue length scales [6,7].
- Disease-positioned mouse models of intestinal regeneration and neoplasia. We have a wide range of cell-specific Cre recombinase models and appropriate driver gene knockout and knock in models. This will allow targeting of mutations to appropriate cells-of-origin. We have optimised a mouse endoscopic colonic wounding model that allows temporal analysis of tissue response to epithelial injury
- RNA and ATAC Seq. We will combine and integrate sequencing technologies with spatial analysis.

Training Opportunities

This project will allow the student to develop their skills with wide and multidisciplinary scientific training. No prior experience is needed. The project will combine wet lab work using mouse models together with spatial biology and deep molecular phenotyping of mouse and human tissue. Students will have the opportunity to learn advanced mouse cancer techniques including endoscopic tissue wounding techniques. Students will work with mathematical collaborators as the biological input to shared analysis of cellular relationships based on

spatial biology dataset interrogation. Students are encouraged to develop their bioinformatic skills to enable them to analyse their own datasets and training for this will be provided.

Key Publications

1) GREMLIN1 disrupts intestinal epithelial-mesenchymal crosstalk to induce a wnt-dependent ectopic stem cell niche via stromal remodelling, Mulholland EM, Belnoue-Davis H, 20 other authors, Leedham SJ. doi: https://doi.org/10.1101/2024.04.28.591245. Bioarchiv 2024, *Under revision at Nature Communications*
2) Integrating diverse statistical methods to analyse stage-discriminatory cell interactions in colorectal neoplasia. Joshua A. Bull, Eoghan J. Mulholland, Joshua W. Moore, Jesús J. Bosque, Bernadette J. Stolz, Joseph Boen, Holly R. Eggington, Hayley L. Belnoue-Davis, Helen Jones, Chandler D. Gatenbee, Alexander R. A. Anderson, Alistair Easton, Peter Todd, Christopher Cunningham, Stephen Taylor, Helen M. Byrne, Simon Leedham. doi: https://doi.org/10.1101/2024.06.02.597010. Bioarchiv, *Under review at Cell Systems*
3) Dynamic and adaptive cancer stem cell population admixture in colorectal neoplasia.Gil-Vasquez E, Nasreddin N, Valbuena GN, Mulholland EJ, Belnoue-Davis HL, Eggington HR, Schenck RO, Wouters VM, Wirapati P, Gilroy K, Lannagan TRM, Flanagan DJ, Najumudeen AK, Omwenga S, McCorry AMB, Easton A, Koelzer VH, East JE, Morton D, Trusolino L, Maughan T, Campbell AD, Loughrey MB, Dunne PD, Tsantoulis P, Huels DJ, Tejpar S, Sansom OJ, Leedham SJ. *Cell Stem Cell.* 2022 Aug 4;29(8):1213-1228
4) Bone Morphogenetic Protein pathway antagonism by Grem1 Regulates Epithelial Cell Fate in Intestinal Regeneration. Koppens MAJ, Davis H, Valbuena GN, Mulholland EJ, Nasreddin N, Colombe M, Antanaviciute A, Biswas S, Friedrich M, Lee L; Oxford IBD Cohort Investigators, Wang LM, Koelzer VH, East JE, Simmons A, Winton DJ, Leedham SJ. *Gastroenterology*. 2021 Apr 2:S0016-5085(21)00579-5. doi: 10.1053
5) Aberrant epithelial *GREM1* expression initiates colonic tumorigenesis from cells outside the stem cell niche. Davis H, Irshad S, Bansal M, Rafferty H, Boitsova T, Bardella C, Jaeger E, Lewis A, Freeman-Mills L, Giner FC, Rodenas-Cuadrado P, Mallappa S, Clark S, Thomas H, Jeffery R, Poulsom R, Rodriguez-Justo M, Novelli M, Chetty R, Silver A, Sansom OJ, Greten FR, Wang LM, East JE, Tomlinson I, Leedham SJ. Aberrant epithelial *GREM1* expression initiates colonic tumorigenesis from cells outside the stem cell niche. *Nature Medicine* 2015; 21(1):62-70.

References

1) Koppens, M. A. J. et al. Bone Morphogenetic Protein Pathway Antagonism by Grem1 Regulates Epithelial Cell Fate in Intestinal Regeneration. Gastroenterology 161, 239-254.e239 (2021). https://doi.org:10.1053/j.gastro.2021.03.052
2) Mulholland, E. et al. GREMLIN1 disrupts intestinal epithelial-mesenchymal crosstalk to induce a wnt-dependent ectopic stem cell niche via stromal remodelling. bioRxiv, 2024.2004.2028.591245 (2024). https://doi.org:10.1101/2024.04.28.591245
3) Vasquez, E. G. et al. Dynamic and adaptive cancer stem cell population admixture in colorectal neoplasia. Cell Stem Cell 29, 1213-1228.e1218 (2022). https://doi.org:10.1016/j.stem.2022.07.008
4) Lee-Six, H. et al. The landscape of somatic mutation in normal colorectal epithelial cells. Nature 574, 532-537 (2019). https://doi.org:10.1038/s41586-019-1672-7

5) Yum, M. K. et al. Tracing oncogene-driven remodelling of the intestinal stem cell niche. Nature 594, 442-447 (2021). https://doi.org:10.1038/s41586-021-03605-0
6) Bull, J. A. et al. Integrating diverse statistical methods to analyse stage-discriminatory cell interactions in colorectal neoplasia. bioRxiv, 2024.2006.2002.597010 (2024). https://doi.org:10.1101/2024.06.02.597010
7) Bull, J. A., Mulholland, E. J., Leedham, S. J. & Byrne, H. M. Extended correlation functions for spatial analysis of multiplex imaging data. bioRxiv, 2023.2006.2020.545678 (2023). https://doi.org:10.1101/2023.06.20.545678

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2.2 RNA Pseudouridylation in cancer

Prof. Chunxiao Song & Prof. Yang Shi

Contact Email Address:

chunxiao.song@ludwig.ox.ac.uk;

yang.shi@ludwig.ox.ac.uk

Project Overview

Cellular RNA is decorated with diverse chemical modifications, which participate in all aspects of RNA biology. The multitude of modifications in RNA adds a new layer to gene regulation, leading to the emerging field of epitranscriptomics. Our research aims to decode the chemical modifications of our genome, transcriptome, and proteome in human health and disease – cancer in particular – and translate this information into diagnostic and therapeutic opportunities that ultimately benefit patients. With our unique expertise in chemical biology and genomic technology, we have developed cutting-edge technologies in epigenetics.

Previously, we developed a bisulfite-free and base-resolution direct sequencing method for DNA epigenetic modifications, and demonstrated its application in circulating cell-free DNA sequencing for early cancer detection (*Nat. Biotechnol*. 2019, *37*, 424, *Sci. Adv.* 2021, *7*, eabh0534, *J. Am. Chem. Soc.* 2023, *145*, 7095). Recently, we developed a novel sequencing method, called BACS, for the most abundant RNA modification pseudouridine (*bioRxiv* 2024.2001.2008.574649, *Nat. Methods* accepted).

We are now applying BACS to uncover the transcriptome-wide distribution of pseudouridine. We will study the role of pseudouridine and pseudouridine synthases (PUS) in cancer, and explore the potential clinical application of pseudouridine in the circulating cell-free RNA for non-invasive cancer diagnostics.

Disease Relevance

Recent studies have increasingly highlighted the emerging role of pseudouridine in cancer. Analysis of public pan-cancer RNA-seq data reveals the global dysregulation of PUS genes in a wide range of cancer types. These findings suggest that the dysregulation of pseudouridine and PUS enzymes is corrected with tumorigenesis. We intend to elusidate the underlying mechanisms. In addition, we also plan to explore the potential clinical application of pseudouridine in the circulating cell-free RNA for non-invasive cancer diagnostics.

Key Technology

2-Bromoacrylamide-Assisted Cyclization Sequencing (BACS), (*bioRxiv* 2024.2001.2008.574649, *Nat. Methods* accepted).

Training Opportunities

- Basic and advanced chemical biology, biochemistry, and molecular biology techniques;
- Knowledge in nucleic acid modifications/epigenetics/epitranscriptomics and clinical diagnostics;
- Sequencing technologies;
- Cell-free RNA sequencing (liquid biopsy);

- Single-cell sequencing and spatial sequencing;
- Bioinformatics and data analysis skills.

Key Publications

1) Xu H, Kong L, Chen J, Moussawi Al K, Chen X, Iqbal A , Wing PAC, Harris JM, Tsukuda S, Embarc-Buh A, Wei G, Castello A, Kriaucionis S, McKeating JA, Lu X, Song CX. (2024). Absolute quantitative and base-resolution sequencing reveals comprehensive landscape of pseudouridine across the human transcriptome. ***bioRxiv*** 2024.2001.2008.574649. (Accepted in ***Nat. Methods***)
2) Liu Y, Siejka-Zielinska P, Velikova G, Bi Y, Yuan F, Tomkova M, Bai C, Chen L, Schuster-Bockler B, Song CX. (2019). Bisulfite-free direct detection of 5-methylcytosine and 5-hydroxymethylcytosine at base resolution. ***Nat. Biotechnol.*** 37, 424-429.
3) Xu H, Chen J, Cheng J, Kong L, Chen X, Inoue M, Liu Y, Kriaucionis S, Zhao M, Song CX. (2023). Modular oxidation of cytosine modifications and their application in direct and quantitative sequencing of 5-hydroxymethylcytosine. ***J. Am. Chem. Soc.*** 145, 7095-7100.
4) Siejka-Zielińska P, Cheng J, Jackson, F, Liu Y, Soonawalla Z, Reddy S, Silva M, Puta L, McCain MV, Culver EL, Bekkali N, Schuster-Böckler B, Palamara PF, Mann D, Reeves H, Barnes E, Sivakumar S, Song CX. (2021). Cell-free DNA TAPS provides multimodal information for early cancer detection. ***Sci. Adv.*** 7, eabh0534.
5) Liu Y, Hu Z, Cheng J, Siejka-Zielinska P, Chen J, Inoue M, Ahmed AA, Song CX. (2021). Subtraction-free and bisulfite-free specific sequencing of 5-methylcytosine and its oxidized derivatives at base resolution. Nat. Commun. 12, 618.

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2.3 New Immune Therapies For Acute Myeloid Leukaemia (AML) And Myeloid Blood Cancers

Prof. Paresh Vyas & Dr. Ricardo Fernandes

Contact Email Address:

paresh.vyas@imm.ox.ac.uk;

ricardo.fernandes@ndm.ox.ac.uk

Project Overview

This exciting project brings together laboratories with established international reputations in haemopoiesis, the biology and treatment of AML (Prof Vyas), protein engineering, antigen-T cell receptor interaction and T cell responses (Dr Fernandes and Dr Dushek). The overall aim of the project is to study in detail how tumour peptides bind HLA-II and how peptide-HLA-II antigens bind to the T cell receptor (TCR) to activate T cell responses. In contrast to well-studied antigen-HLA-I binding and T cell activation, much still remains to be understood about functionally important peptide-HLA-II (pHLA)-TCR interactions. The work will lead to development of novel TCR-based therapies for the most common aggressive adult human leukaemia, AML.

By studying AML patients cured from an allogeneic stem and immune cell transplant, we have identified novel pHLA antigen targets on patient AML cells and the T cell receptors that recognise those targets. Interestingly, the bulk of these pHLA targets are HLA-II restricted and correspondingly elicit CD4 cytotoxic T cell responses. This project will focus on a limited number of pHLA antigens we have identified and characterise the detailed mechanisms of pHLA-II-TCR interactions through three aims:

1) ***Characterisation of HLA-II binding to alloreactive peptide.*** Using molecular biology and protein engineering methods the applicant will express soluble pHLA-II with varying peptide lengths and study the biophysical properties of peptide-HLA-II binding and compare this to computationally derived prediction of peptide-HLA-II binding. By mutating peptide resides the applicant can identify which peptide residues are critical for binding. This work will be combined with structural studies in Aim 3.
2) ***Characterisation of TCR binding and crossreactivity to pHLA-II antigen.*** Using molecular biology and protein engineering methods the applicant will express soluble TCR and test the biophysical properties of binding to pHLA-II to TCR. By mutating TCR resides the applicant can identify which TCR residues are critical for binding. If time permits, the applicant will generate a large, unbiased, peptide-MHC library for yeast display to determine the TCR crossreactivity of wild-type and engineered TCRs. Finally, for select TCRs that bind pHLA-II the mode of T cell activation and need for co-stimulation will be studied. This work will be combined with structural studies in Aim 3.
3) ***Structural studies of TCR-PHLA-II binding***.The data in Aims 2 and 3 will be greatly strengthened by structural studies of pHLA-TCR interaction. The applicant will take select soluble p-HLA-II antigens and cognate TCRs from aims 2 and 3 collaborate with a structural biology group to enable them to make crystals for structural studies using a range of structural resolution methods.

The combination of Aims 1-3 will provide detailed insight into the mechanisms of p-HLA-II binding and the binding of pHLA-II antigens with TCRs.

Disease Relevance

This project aims to identify the optimal pHLA antigens and TCRs for either TCR engager therapy or TCR T-cell therapies for AML and myeloid cancers. There are ~ 44 000 patients diagnosed with AML annnually in the US and EU and another 42 000 with other myeloid cancers.

Key Technology

- Protein engineering: complex molecular cloning, protein expression, protein purification,
- Biophysical methods of measuring protein-protein interaction including surface plasma resonance.
- Complex multi-colour flow cytometric analysis and FACS-sorting. All students will independently use flow analysers and sorters.
- Lentiviral and adeno-associated viral transduction of immune cells.
- In vitro and in vivo assays of T cell function including biochemical analysis of TCR signalling.
- Crispr gene editing of cell lines and primary cells.
- Computational analysis including exposure to coding using Python and R.
- NGS libraries for RNA-seq, TCR sequencing, single cell genotyping+ ATAC-seq in single cells and highly purified cell populations.

Training Opportunities

The DPhil student will be trained in: (i) fundamental aspects of immunology and specifically pHLA interactions and the binding of pHLA to TCR; (2) molecular biology, protein engineering and biophysical measurements of protein-protein interaction: (3) computational biology (4) structural biology. The training will be focussed on specific skill sets that are critical for developing immune therapies.

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

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

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

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

Key Publications

1) Jakobsen NA, Turkalj S, Zeng AGX, Stoilova B, Metzner M, Rahmig S, Nagee MS, Shah S, Moore R, Usukhbayar B, Salazar MA, Gafencu GA, Kennedy A, Newman S, Kendrick BJL, Taylor AH, Afinowi-Luitz R, Gundle R, Watkins B, Wheway K, Beazley D, Murison A, Aguilar-Navarro AG, Flores-Figueroa E, Dakin SG, Carr AJ, Nerlov C, Dick JE*, Xie SZ*, **Vyas P*.** Selective advantage of mutatant stem cells in human clonal hematopoiesis is associated with attenuated response to inflammation and aging. *Co-last authors**. Cell Stem Cell.** Jun 19:S1934-5909(24)00207-8. doi: 10.1016/j.stem.2024.05.010. **(2024).** PMID: 38917807.
2) Aksoz M, Gafencu G, Stoilova B, Buono M, Zhang Y, Turkalj S, Meng Y, Jakobsen NA, Metzner M, Clark SA, Bevridge R, Thongjuea S, Vyas P*, Nerlov C*. Hematopoietic stem cell heterogeneity and age-associated platelet bias are evolutionarily conserved. *Co-last authors. Science Immunology. Aug 23;9 (98)eadk3498.doi: oi: 10.1126/sciimmunol.adk3469. Epub 2024 Aug 23. (2024). PMID: 39178276.
3) Versluis J, Metzner M, Wang A, Gradowska P, Thomas A, Jakobsen NA, Kennedy A, Moore R, Boertjes E, Vonk CM, Kavelaars FG, Rijken M, Gilkes A, Schwab C, Beverloo HB, Manz M, Visser O, Van Elssen CHMJ, de Weerdt O, Tick LW, Biemond BJ, Vekemans MC, Freeman SD, Harrison CJ, Cook JA, Dennis M, Knapper S, Thomas I, Craddock C, Ossenkoppele, Lowenberg B, Russell N, Valk PJM, Vyas P. Risk stratification in older intensively treated patients with acute myeloid leukemia. *Co-last authors. Journal of Clnical Oncology 2024 Sep 4:JCO2302631. doi: 10.1200/JCO.23.02631. Online ahead of print. (2024). PMID: 39231389.
4) Turkalj S, Jakobsen NA, Groom A, Metzner M, Giva SG, Gür ER, Usukhbayar B, Salazar MA, Hentges LD, Mickute G, Clark K, Sopp P, Davies JOJ, Hughes JR,Vyas P. GTAC enables parallel genotyping of multiple genomic loci with chromatin accessibility profiling in single cells. Cell Stem Cell. May 4;30(5):722-740.e11. doi: 10.1016/j.stem.2023.04.012. (2023) PMID: 37146586.
5) Quek L, David M, Kennedy A, Metzner M, Amatangelo M, Shih A, Stoilova B, Quivoron C, Heiblig M, Willekens C, Saada V, Peniket A, Bernard O, Agresta S, Yen K, MacBeth K, Stein E, Levine R, De Botton S, Thakurta A, Penard-Lacronique V and Vyas P. Clonal Heterogeneity in Differentiation Response and Resistance to the IDH2 inhibitor Enasidenib in Acute Myeloid Leukemia. Nature Medicine. Aug 24(8):1167-1177. Doi: 10.1038/s41591-018-0115-6. (2018). PMID: 30013198.

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2.4 Mechanisms of genomic and epigenomic instability in TP53 mutant AML

Prof. Paresh Vyas, Prof. KJ Patel & Prof. Ross Chapman

Contact Email Address:

paresh.vyas@imm.ox.ac.uk;

kj.patel@imm.ox.ac.uk;

ross.chapman@imm.ox.ac.uk

Project Overview

Project Description:

Genetic changes (point mutations or copy number abnormalities) that disrupt function of TP53 occur in ~ 10% of patients with Acute Myeloid Leukaemia (AML), the most aggressive adult leukaemia. *TP53* mutant (*TP53*m) AML has a prognosis of only ~ 6 months. *TP53*m is often associated with complex chromosomal changes arising from chromothripis. The mechanisms that trigger chromothripis and lead to the selection of specific chromosomal rearrangements in *TP53*m AML is unclear. Understanding these mechanisms may lead to novel therapeutic approaches for this AML subtype for which there is high unmet need.

Background:

We have spent the last decade characterising in great detail, normal human haeemopietic stem/progenitor biology (Karamitros et al Nature Immunology 2018 and Aksoz et al Science Immunology 2024). Blood stem progenitor cells acquire genetic and epigenetic changes gradually transform them into leukaemia propagating stem cell (Goardon et al Cancer Cell 2011; Quek et al Journal of Experimental Medicine 2016; Labuhn et al Cancer Cell 2019; Genua et al Cancer Cell 2020; Turkajl et al Cell Stem Cell 2023; Jakobsen et al Cell Stem Cell 2024). More recently, we have developed quantitative models to detect selection and infer the clonal stem/progenitor dynamics (age of clones, clonal division rates, stem cell numbers) (Korber et al 2024). Over the last 3 years we have been characterising a cohort of 45 *TP53m* AML (bulk whole genome sequencing, single cell multi-omic RNA, ATAC-Seq and immunophenotyping profiling). In these AML samples there are a hierarchy of recurrent driver mutations and complex chromosomal changes that arise by chromothripsis and that are selected to provide clonal advantage. We wish to understand the mechanisms by chromothripsis occurs and the mechanisms underlying the selection of genetic (copy number changes) and corresponding chromatin changes.

Disease Relevance

TP53 mutation is one of the most common cancer-causing mutations. TP53 mutant AML has one of the worst prognosis within AML. The project aims to understand the mechanisms of complex karyotype changes in TP53 mutant AML and the how they lead to clonal selection. Ultimately, the aim of this work is to identify therapeutic vulnerabilities in this cancer of high unmet need.

Key Technology

- Advanced flow cytometry including FACS sorting

- In vitro and in vivo CRISPR/Cas9-based genome editing screens and library screening technologies
- Advanced mouse genetics
- Bioinformatics
- Candidates will also have exposure to mathematical modelling and machine learning

Training Opportunities

Training will be provided in studying genome instability leading to chromothripsis, HSC and progenitor biology that are the cellular template for selection. From a methods point of view the applicant will conduct and be given experience and training in the wet lab molecular and cellular techniques and advanced computational analysis including advanced flow cytometry including FACS sorting, in vitro and in vivo CRISPR/Cas9-based genome editing screens and library screening technologies, advanced mouse genetics and bioinformatics. Candidates will also have exposure to mathematical modelling and machine learning.

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

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

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

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

Key Publications

1) Jakobsen NA, Turkalj S, Zeng AGX, Stoilova B, Metzner M, Rahmig S, Nagee MS, Shah S, Moore R, Usukhbayar B, Salazar MA, Gafencu GA, Kennedy A, Newman S, Kendrick BJL, Taylor AH, Afinowi-Luitz R, Gundle R, Watkins B, Wheway K, Beazley D, Murison A, Aguilar-Navarro AG, Flores-Figueroa E, Dakin SG, Carr AJ, Nerlov C, Dick JE*, Xie SZ*, Vyas P*. Selective advantage of mutatant stem cells in human clonal hematopoiesis is associated with attenuated response to inflammation and aging. *Co-last authors. Cell Stem Cell. Jun 19:S1934-5909(24)00207-8. doi: 10.1016/j.stem.2024.05.010. (2024). PMID: 38917807.
2) Aksoz M, Gafencu G, Stoilova B, Buono M, Zhang Y, Turkalj S, Meng Y, Jakobsen NA, Metzner M, Clark SA, Bevridge R, Thongjuea S, Vyas P*, Nerlov C*. Hematopoietic stem cell heterogeneity and age-associated platelet bias are evolutionarily conserved. *Co-last

authors. Science Immunology. Aug 23;9 (98)eadk3498.doi: 10.1126/sciimmunol.adk3469. Epub 2024 Aug 23. (2024). PMID: 39178276.

3) Versluis J, Metzner M, Wang A, Gradowska P, Thomas A, Jakobsen NA, Kennedy A, Moore R, Boertjes E, Vonk CM, Kavelaars FG, Rijken M, Gilkes A, Schwab C, Beverloo HB, Manz M, Visser O, Van Elssen CHMJ, de Weerdt O, Tick LW, Biemond BJ, Vekemans MC, Freeman SD, Harrison CJ, Cook JA, Dennis M, Knapper S, Thomas I, Craddock C, Ossenkoppele, Lowenberg B, Russell N, Valk PJM, Vyas P. Risk stratification in older intensively treated patients with acute myeloid leukemia. *Co-last authors. Journal of Clnical Oncology 2024 Sep 4:JCO2302631. doi: 10.1200/JCO.23.02631. Online ahead of print. (2024). PMID: 39231389.
4) Turkalj S, Jakobsen NA, Groom A, Metzner M, Giva SG, Gür ER, Usukhbayar B, Salazar MA, Hentges LD, Mickute G, Clark K, Sopp P, Davies JOJ, Hughes JR,Vyas P. GTAC enables parallel genotyping of multiple genomic loci with chromatin accessibility profiling in single cells. Cell Stem Cell. May 4;30(5):722-740.e11. doi: 10.1016/j.stem.2023.04.012. (2023) PMID: 37146586.
5) Quek L, David M, Kennedy A, Metzner M, Amatangelo M, Shih A, Stoilova B, Quivoron C, Heiblig M, Willekens C, Saada V, Peniket A, Bernard O, Agresta S, Yen K, MacBeth K, Stein E, Levine R, De Botton S, Thakurta A, Penard-Lacronique V and Vyas P. Clonal Heterogeneity in Differentiation Response and Resistance to the IDH2 inhibitor Enasidenib in Acute Myeloid Leukemia. Nature Medicine. Aug 24(8):1167-1177. Doi: 10.1038/s41591-018-0115-6. (2018). PMID: 30013198.

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2.5 Modulating inhibitory receptor signaling to potentiate T cell function in cancer

Dr. Ricardo A. Fernandes & Prof. Omer Dushek

Contact Email Address:

ricardo.fernandes@ndm.ox.ac.uk;

omer.dushek@path.ox.ac.uk

Project Overview

Background

The T cell response against pathogens, tumours, and self relies on integrating signals from diverse receptors, including the T cell receptor, co-stimulatory receptors, and inhibitory receptors (IRs). Inhibitory receptors counterbalance positive signals, suppressing T cell responses. A feature of IRs is the presence of signalling motifs in their cytoplasmic tail, which attenuate T cell responses. While antibody blockade, which limits IR/ligand interactions, represents a significant advancement in antitumour therapy, only a small fraction of patients benefit from this approach. Moreover, the mechanistic basis whereby IRs damped T cell responses is still poorly understood, and a systematic comparison of the potency, mediators, and targets of various clinically relevant IRs has not yet been undertaken. This proposal seeks to deepen our understanding of IR signalling and generate novel workflows to potentiate T cell responses in cancer.

Research objectives and proposed outcomes

Regulation of T cell signalling by immune checkpoints such as PD-1 and CTLA-4 has been at the centre of recent breakthroughs in cancer immunotherapy. Signalling by PD-1 and CTLA-4 reduces T cell activity and contributes to an “exhausted” phenotype, severely compromising antitumor responses. In the case of PD-1, binding to PD-L1/2 triggers the tyrosine phosphorylation of signalling motifs and results in the recruitment of cytosolic phosphatases such as SHP1/2, which in turn reduces TCR and CD28 signalling. Strikingly, signalling by several immune receptors relies on the Tyr phosphorylation of signalling motifs. We hypothesise that tonic receptor phosphorylation and sustained signalling by ‘ligand-experienced’ receptors impact T cell function and resist classic antibody blockade. To address this issue, we engineered a bi-specific molecule to recruit CD45, an abundant and promiscuous receptor tyrosine phosphatase, within close proximity of PD-1 (Fig. 1). In this approach, named Receptor Inhibition by Phosphatase Recruitment (RIPR), the phosphatase domain of CD45 acts intracellularly, in *cis,* on the p-Tyr residues of the PD-1 signalling motif, thus inhibiting sustained signalling.

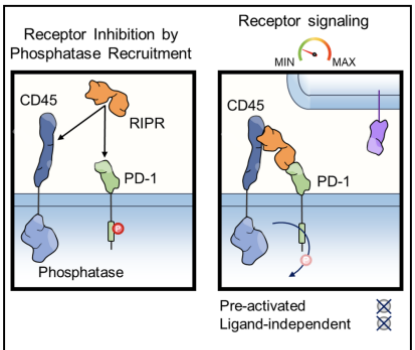

Fig. 1 | Receptor Inhibition by Phosphatase Recruitment. RIPR combines checkpoint blockade with CD45 recruitment. CD45 recruitment dephosphorylates receptors such as PD-1.

Aim 1: Determine the relative signalling potency of IRs in T cells

IRs have a distinct organisation of inhibitory motifs in their cytoplasmic domain, ranging from non-conventional single tyrosine (Tyr) motifs to a combination of two or more “classic” inhibitory motifs like ITIMs. We will perform systematic screens to determine the ability to suppress T cell function by various IRs, including PD-1, TIGIT, BTLA, TIM-3, CTLA-4, LAIR-1, ILT-2 and ILT-4. In addition, we will compare IR signalling potencies in the presence and absence of IR-ligand binding using surrogate receptor-ligand pairs.

Aim 2: Development of RIPR-based molecules to inhibit inhibitory receptors

Bispecific diabodies that recruit CD45 phosphatase to IRs, such as PD-1 and CTLA-4, were found to potentiate T cell responses. We aim to extend this concept to target additional IRs, including BTLA, TIGIT and TIM-3, which have been implicated in suppressing antitumour responses. Moreover, we will systematically test the potency of newly generated RIPR proteins using various anti-CD45 nanobodies. This information will be used to guide the design of future antagonists of checkpoint receptor signalling with strong potential for therapeutic applications.

Disease Relevance

We expect that the described approach will establish a rapid and facile method to systematically probe the contribution of inhibitory receptors in suppressing T cell effector functions. This information will enable the identification of new targets and guide the development of IR therapeutics in cancer. We anticipate the next stage of immunotherapy development to include new molecules that exploit specific aspects of the mechanisms involved in receptor signalling. The RIPR approach may offer a new avenue to directly target receptor phosphorylation and shut down inhibitory receptor signalling with a strong potential for being used to target various surface receptors found in distinct immune cell populations.

Key Technology

- T cell signalling
- Protein engineering
- Antibody discovery
- Biophysical characterization
- Cell signalling
- T cell activation
- T cell engineering

Training Opportunities

The candidate will receive training in molecular biology, protein design and expression, biophysical characterisation of protein interactions and various cellular assays. Moreover, the candidate will be trained in protein engineering, library design and selection using yeast display. T cell signalling assays will be used to determine the activity of newly generated RIPR molecules, providing an opportunity for training in flow cytometry and MS, among others. This training will allow the candidate to drive fundamental and applied research in academia and industry. The candidate will have full access to the facilities and resources available within the Department and the broader community at the University of Oxford.

Key Publications

1) Fernandes RA, Su L, Nishiga Y, Ren J, Bhuiyan AM, Ali LR, Majzner R, Ohtsuki S, Rietberg SP, Yang X, Picton L, Savvides CS, Mackall, CL, Sage J, Dougan M, Garcia KC. Immune receptor inhibition through enforced phosphatase recruitment. (2020) Nature, Oct;586(7831):779-784
2) Fernandes RA*, Ganzinger KA*, Tzou J, Jonsson P, Lee SF, Palayret M, Santos AM, Chang VT, Macleod C, Lagerholm BC, Lindsay AE, Dushek O, Tilevik A, Davis SD, Klenerman D. A cell-topography based mechanism for ligand discrimination by the T-cell receptor. (2019) Proc Natl Acad Sci U S A. Jul; 116(28), 14002-14010
3) Chang VT*, Fernandes RA*, Ganzinger KA*, Lee SF*, Siebold C, McColl J, Jönsson P, Palayret M, Harlos K, Coles CH, Jones EY, Lui Y, Huang E, Gilbert RJ, Klenerman D, Aricescu AR, Davis SJ. Initiation of T cell signaling by CD45 segregation at 'close contacts'. (2016) Nat Immunol. May;17(5):574-82

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2.6 Discovery of potent agonist peptides for tumour-reactive T cells

Dr. Ricardo A. Fernandes & Prof. Tao Dong

Contact Email Address:

ricardo.fernandes@ndm.ox.ac.uk;

Tao.dong@ndm.ox.ac.uk

Project Overview

Abstract

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

Project Overview

Identifying antigens recognised by the TCR is challenging given the extreme diversity of the three individual components involved: peptide antigens, TCR and MHC. We aim to identify peptides, neoantigens and mimotopes, recognised by the TCR of clonally expanded CD8+ effector T cells in tumour settings (Fig. 1). To this end, we will engineer large (> 109) peptide-MHC libraries to be displayed at the surface of yeast cells, after which we will use an affinity-based screen to identify peptides recognised by TCRs of interest. This affinity-based approach will be complemented by a functional screen using an engineered system in mammalian cells. In this recently developed approach, the peptide-MHC library is fused to a CAR-like signalling module and displayed in T cells. This functional-based selection hijacks the unique sensitivity and specificity of the CD28/CD3 signalling modules to report on a productive TCR/pMHC interaction. Sorting of cells based on the upregulation of activation markers such as CD69 and CD25 will be used to isolate agonist peptides of different potency.

The combination of affinity- and activity-based selections will guide the identification of potent agonist mimotopes, self-peptides or neoantigens using custom-built algorithms to rank closely related wild-type peptides. The identification of peptides recognised by tumour-reactive T cells will facilitate their expansion and detection using peptide-MHC molecules. Moreover, following isolation or activation with agonist peptides, tumour-reactive T cells will be characterised using single-cell transcriptomics and proteomics, for example. Agonist peptide identification combined with single-cell sequencing and quantitative proteomic analysis of relevant T cells will expand our current understanding of the role of diverse T cell subsets during an anti-tumour immune response. Furthermore, the discovery of disease-related agonist peptides opens the possibility to modulate T cell responses by peptide immunisation, an essential first step towards achieving in vivo expansion and activation of

tumour-specific T cells. This research plan thus aims to contribute towards the development of relevant immunotherapies in cancer settings and a better understanding of T cell function.

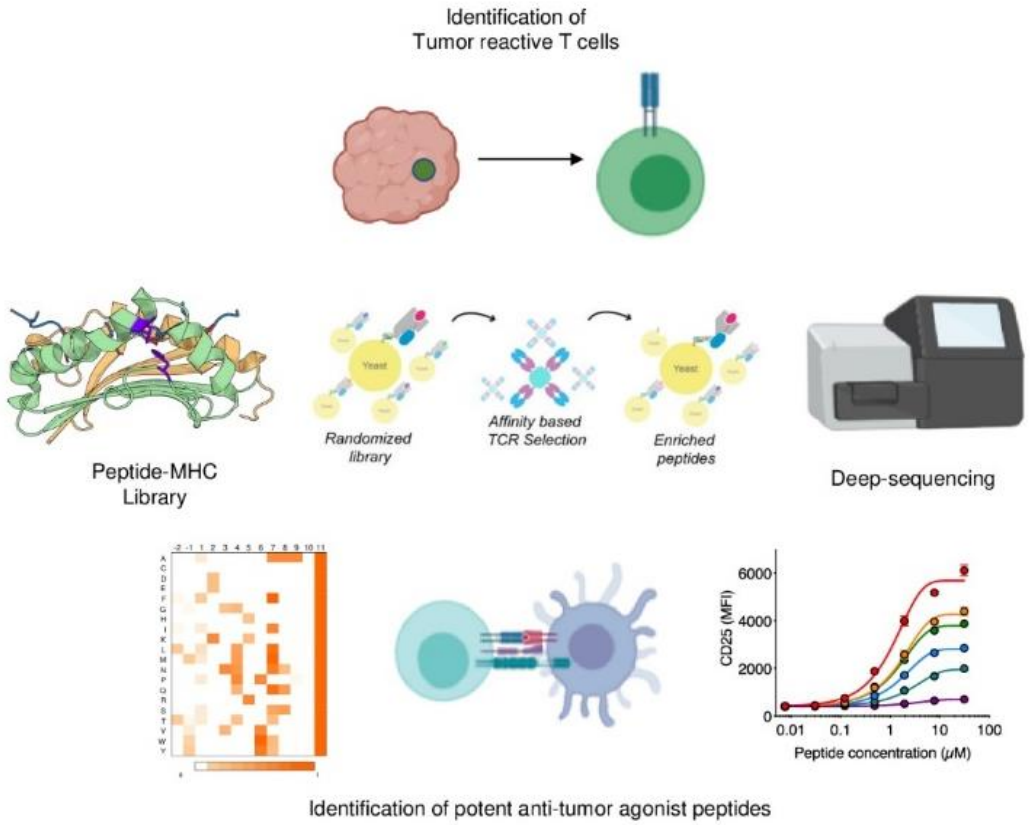

Figure 1. General overview of the experimental approach to discover peptide antigens to elicit robust anti-tumor T cell responses.

Disease Relevance

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

Key Technology

- Protein engineering
- Yeast display
- Antigen discovery
- Biophysical analysis of protein-protein interactions (including SPR)

- Flow cytometry; deep-sequencing (NGS)
- T cell functional assays using cell lines and primary cells
- Cell engineering, TCR-T

Training Opportunities

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

Key Publications

1) Gee MH, Han A, Lofgren SM, Beausang JF, Mendoza JL, Birnbaum ME, Bethune MT, Fisher S, Yang X, Bingham DB, Sibener LV, Fernandes RA, Velasco A, Baltimore, D, Schumacher TN, Khatri P, Quake SR, Davis MM, Garcia KC. Antigen identification for orphan T cell receptors expressed on tumor-infiltrating lymphocytes. (2018) Cell. Jan 25;172(3):549-563.e16.
2) Sibener LV, Fernandes RA, Kolawole EM, Carbone CB, Liu F, McAffee D, Yang D, Su DF, Yu D, Dong S, Gee MG, Jude KM, Birnbaum ME, Goddard WA, Davis MM, Groves JT, Heath JR, Evavold BD, Vale RD, Garcia KC. Isolation of a structural trigger required for TCR signaling from analysis of non-stimulatory peptide-MHC ligands. (2018) Cell. Jul; 174 (3), 672-687. e27.
3) Fernandes RA*, Li C*, Wang G, Yang X, Savvides CS, Glassman CR, Dong S, Luxemberg E, Sibener LV, Birnbaum ME, Benoist C, Mathis D, Garcia KC. Discovery of surrogate agonists for visceral fat Treg cells that modulate metabolic indices in vivo. (2020) eLife. Aug; 9:e58463
4) Sušac L, Vuong MT, Thomas C, von Bülow S, O'Brien-Ball C, Santos AM, Fernandes RA, Hummer G, Tampé R, Davis SJ. Structure of a fully assembled tumor-specific T cell receptor ligated by pMHC. Cell. 2022 Aug 18;185(17):3201-3213.e19.
5) Yang X, Garner LI, Zvyagin IV, Paley MA, Komech EA, Jude KM, Zhao X, Fernandes RA, Hassman LM, Paley GL, Savvides CS, Brackenridge S, Quastel MN, Chudakov DM, Bowness P, Yokoyama WM, McMichael AJ, Gillespie GM, Garcia KC. Autoimmunity-associated T cell receptors recognize HLA-B*27-bound peptides. Nature. 2022 Dec;612(7941):771-777.

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2.7 Investigating Epigenetic and Epitranscriptomic regulation in Acute Myeloid Leukemia (AML)

Prof. Yang Shi & Prof. Stepan Constantinescu

Contact Email Address:

yang.shi@ludwig.ox.ac.uk;

stepan.constantinescu@ludwig.ox.ac.uk

Project Overview

Epigenetic and epitranscriptomic changes constitute pivotal factors driving the development and progression of various human cancers, including leukemia. Recent advancements in cancer research underscore the significance of these alterations in shaping complex regulatory networks, not only contributing to the initiation and progression of leukemia but also developing resistance to therapy.

In 2004, our laboratory identified the first histone demethylase, LSD1, whose role was later revealed to be crucial in the initiation and development of several cancers, including leukemia. More recently, our group has unveiled novel RNA modifier enzymes with potential roles in leukemia, presenting exciting opportunities for therapeutic targeting. This D.Phil project aims to unravel the intricacies of epigenetic and epitranscriptomic changes in leukemia, identifying novel therapeutic targets to advance therapy for leukemia patients.

Disease Relevance

This project focuses on Acute Myeloid Leukemia (AML) and secondary AML, which present an unmet medical need.

Key Technology

Develop proficiency in essential techniques, including cell culture and handling primary cells, cloning, CRISPR/Cas9 genome editing, qRT-PCR, virus packaging, mammalian cell transduction, chromatin immunoprecipitation, and chromosome-conformation-capture.

Training Opportunities

The collaborative environment in our lab provides a rich learning experience, enhancing skills in state-of-the-art technologies.

This includes techniques for the analysis of gene regulation (ATAC-seq, ChIP-seq, chromosome-conformation- capture, RNA-seq), advanced molecular biology, genome editing, and computational biology, etc. Trainees participate in weekly internal seminars and monthly external seminars within our Institute, which keep students abreast of recent advancements in cancer research and related fields and provide opportunities for enhancing presentation skills.

Key Publications

1) Zee, B.M., Poels, K.E., Yao, C.H., Jacobus, W.D., Senior, E., Endress, J.E., Jambhekar, A., Lovitch, S.B., Ma, J.X., Dhall, A., Harris, I.S., Blanco, A., Skykes, D.B., Haigis, M.C., Michor, F., Licht, J.D., and Shi, Y. Combined epigenetic and metabolic treatments overcome differentiation blockade in AML. iScience, 2021. May 25;24(6):102651. doi: 10.1016/j.isci.2021.102651. eCollection 2021 Jun 25. PMID: 34151238

2) Blanco, AM*, +., Skyes, DB*., Gu, L*., Wu, MJ., Petroni, R.,Karnik, R., Wawer, M., Rico, J., Li, HT., Jacobus, WD., Jambhekar, A., Cheloufi, S., Meissner, A., Hochedlinger, K., Scadden, DT+., and Shi, Y+. Chromatin state barriers enforce an irreversible mammalian cell fate decision.
   a) Cell Rep. 2021, Nov 9;37(6):109967. doi: 10.1016/j.celrep.2021.109967.
   b) *Equal contributions; +Co-correspondence.

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3. Infection, Inflammation & Disease

3.1 Towards a mechanistic understanding of humoral immunity

Prof. Oliver Bannard & Prof. Simon Davis

Contact Email Address:

oliver.bannard@ndm.ox.ac.uk;

simon.davis@imm.ox.ac.uk

Project Overview

Our lab seeks to unravel the mechanisms controlling the establishment of antibody-mediated (humoral) immunity during infections and following immunization. We have a particular interest in germinal centres (GCs).

Germinal centres (GCs) are specialised structures that form in secondary lymphoid tissues during immune responses. Here, B cells undertake a remarkable process known as antibody affinity maturation – this involves them somatically mutating their immunoglobulin genes and undergoing sequential rounds of competitive affinity-based selection, leading to progressive increases in antibody binding strength. At their best, GCs can reshape antibodies with negligible or very low starting affinity into highly potent and protective molecules, such as broadly neutralizing antibodies to HIV. This allows responses to adapt to each new pathogen encountered. Following their maturation, some GC B cells are chosen to differentiate, giving rise to long-lived memory B cells and plasma cells. By establishing how antibody affinity maturation and B cell differentiation are regulated, we aim to inform the rational design of better vaccines. We are also motivated by the fascinating and unique nature of the biology involved.

DPhil projects in the Bannard lab are centred around deciphering the fundamental biological processes underpinning the selection in GCs and the development of humoral immunity. We will design specific projects based around shared interests of the applicant and lab, considering what are the most pressing issues at the time. Recent DPhil student projects have involved providing a first snapshot of the plasma cells generated in GCs during infection (Sprumont et al 2023, Cell, PMID 37951212), identifying a new “non-binary” GC selection model (Long et al. 2022, Science Immunology PMID 35275753) and defining a previously unknown negative selection process that deletes B cells that have acquired damaging mutations (Stewart et al. 2018, Immunity PMID 30231983). Following their death, such cells are rapidly cleared by a unique macrophage subset called tingible body macrophages, which we recently demonstrated the developmental origin and functional behaviours of (Grootveld et al. 2023, Cell, PMID 36868219).

Disease Relevance

Our immune systems allow us to co-exist alongside commensal and pathogenic organisms without succumbing to infection. Antibodies play a major role in this, playing critical roles in neutralising pathogen functions and targeting them for destruction. Our research is aimed at understanding the formation and regulation of antibody responses, and thereby informing the development of better vaccines and immunotherapies. In the case of the former, important questions include understanding what determines the immune focussing of responses (so they can be targeted in desirable ways), how one can shepherd antibody affinity maturation

pathways is desirable ways (to allow guidance of complex antibody maturation pathways, e.g. to HIV) and what determines antibody response magnitude and duration (both factors that are limiting, especially for vaccines requiring sterilising immunity). The regulation of humoral immunity is also directly relevant for autoimmune and inflammatory disorders where responses may be excessive or misguided.

Key Technology

- Complex genetically modified in vivo models
- Crispr/Cas9-mediated organism modifications
- Single cell antibody cloning
- Surface plasmon resonance
- High dimensional flow cytometry
- Flow cytometric sorting
- Single cell RNA-seq
- Live tissue imaging (2 photon microscopy)
- In tissue photoconversion (e.g. KikGR and photoactivatable GFP)
- Molecular cloning
- Genetic fate-mapping
- Spatial analysis.

Training Opportunities

We study immune cells within their native tissue contexts by using complex in vivo genetically modified models, cutting edge ex vivo approaches (e.g., single cell RNA-seq, high end flow cytometry, confocal microscopy, single B cell antibody cloning), and live in situ imaging to visualise and track cell behaviour in real time (e.g. 2-photon microscopy). Our studies are performed in the context of infections (e.g., Influenza A virus) and immunisations. As such, students can expect receive sound intellectual and practical science training.

Key Publications

1) Sprumont A., Rodrigues A., McGowan S., Bannard C., Bannard O. 2023. Germinal centers output clonally diverse plasma cell populations expressing high- and low- affinity antibodies. Cell, 186(25):5486-5499.
2) Grootveld A.K.*, Kyaw W., Panova V., Lau A., Ashwin E., Seuzaret G., Dhenni R., Bhattacharyya N., Khoo W.H., Biro M., Mitra T., Meyer-Hermann M., Bertolino P., Tanaka M., Hume D., Croucher P., Brink R., Nguyen A., Bannard O.*, Phan T.G.*. 2023. Apoptotic cell fragments locally activate tingible body macrophages in the germinal center. Cell, 186(6): 1144-1161. *Corresponding authors
3) MacLean AJ, Richmond N, Koneva L, Attar M, Medina CAP, Thornton EE, Gomes AC, El-Turabi A, Bachmann MF, Rijal P, Tan TK, Townsend A, Sansom SN, Bannard O*, Arnon TI*. (2022). Secondary influenza challenge triggers resident memory B cell migration and rapid relocation to boost antibody secretion at infected sites. Immunity, 55(4):718-733. *Corresponding authors.

4) Long Z., Phillips P., Radtke D., Meyer-Hermann M., Bannard O. Competition for refuelling rather than cyclic re-entry initiation evident in germinal centers. Science Immunology. 11;7(69):eabm0775
5) Stewart, I., Radtke, D., Phillips, B., McGowan, S., Bannard, O. (2018). Rapid B cell receptor turnover in germinal center dark zones facilitates the deletion of cells acquiring damaging mutations. Immunity. 49:477-489.

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3.2 Exploiting electronic health records to infection management and optimise antimicrobial use

Prof. A. Sarah Walker, Prof. David Eyre & Dr. Bernadette Young

Contact Email Address:

sarah.walker@ndm.ox.ac.uk;

david.eyre@bdi.ox.ac.uk;

bernadette.young@ndm.ox.ac.uk

Project Overview

Large-scale electronic health record data provide the potential to answer a far greater number of questions about infection management than traditional epidemiological studies using questionnaires. Their volume and scale are continuously increasing as larger and larger amounts of healthcare data are linked and de-identified for research, for example from primary as well as secondary care. Examples and challenges include：

- Can we identify a wider range of risk factors for infection to target interventions? The sheer number of factors that could be considered, many with substantial amounts of missing data, poses challenges to traditional epidemiological approaches. There are similarities to challenges faced by Genome Wide Association Studies, in terms of both number of factors and potential strong associations between them (for example diabetes/pre-diabetes may be reflected in diagnostic codes, procedure codes and lab test results such as HbA1c). As well as more traditional approaches [1], a recent novel statistical analysis approach called 'doublethink' [2] has been proposed to bridge this gap, with an exciting application to investigating associations with COVID-19 hospitalisation in the Biobank database [3]. These methods could be applied to a range of microbiologically and/or syndromically defined infections to identify novel populations to target to reduce infection risks.
- Can we work out how best to use diagnostic tests for infection, widely considered to be a key tool to improve antimicrobial stewardship, in the real-world - in what patient populations are they and should they be used, how often, and what are their ultimate effects on both antimicrobials prescribed and patient outcomes? Unlike a drug which acts directly on an organism, diagnostics work indirectly through changing prescribing behaviours, and can be used at multiple different places in a patient's prescribing pathway [4]. Further, there are increasing numbers of molecular based diagnostics which aim to identify which of multiple viral or bacterial pathogens might be causing an infection, and these are relatively high cost. There is a real risk that tests just become used more and more, without actually changing anything. Despite this, little epidemiological research has explored exactly how and when these diagnostic tests are being used in the real-world.

DPhil projects will exploit an existing large data-warehouse of de-identified individual patient data, called the Infections in Oxfordshire Database (https://oxfordbrc.nihr.ac.uk/research-themes/modernising-medical-microbiology-and-big-infection-diagnostics/iord-about/), including admissions, prescriptions, laboratory and microbiological (diagnostic) test results. There will be opportunities to learn how to manage and use large-scale electronic health record data, and apply novel causal epidemiological methods, as well as traditional statistical and machine learning approaches.

This project would suit a student with an interest in infections and antimicrobial usage who wishes to gain experience in design of epidemiological studies, a range of quantitative methods and in use of electronic health records to answer real-world questions.

Disease Relevance

The proposed projects are directly relevant to the management and treatment of infections, whether these are confirmed through microbiological testing or identified syndromically. Supervisors include Consultants in Infection/Microbiology and the lead for Infection Prevention and Control at the Oxford University Hospitals NHS Foundation Trust, ensuring that the project is tightly linked to real-world clinical questions and practice.

Key Technology

- Linked and de-identified electronic health records
- Statistical and epidemiological analysis

Training Opportunities

Specific training will be in:

- A wide range of statistical and epidemiological methods, including (depending on student's interest) causal epidemiological methods such as target trial emulation
- Use of large-scale multimodal electronic health record data
- Working closely with clinicians from NHS hospitals and learning how to address real-world problems that are also technically exciting and challenging
- Antimicrobial stewardship and microbiology
- Attending relevant specialised training courses will be encouraged

The student will join a dynamic team of around 30 DPhil students and post-docs with expertise in biostatistics, epidemiology, machine learning, infectious diseases, microbiology, molecular biology and bioinformatics, providing opportunities for skills and career development, both in terms of biostatistics/epidemiology and more broadly in terms of research careers. Our group, located primarily at the John Radcliffe Hospital, has strong inter-disciplinary links with national and international collaborators and public health agencies, and represents a unique opportunity promoting pathways to research translation in infectious diseases.

Key Publications

1) https://pubmed.ncbi.nlm.nih.gov/34927119/
   Monitoring populations at increased risk for SARS-CoV-2 infection in the community using population-level demographic and behavioural surveillance
   Emma Pritchard, Joel Jones, Karina-Doris Vihta, Nicole Stoesser, Philippa C Matthews, David W Eyre, Thomas House, John I Bell, John N Newton, Jeremy Farrar, Derrick Crook, Susan Hopkins, Duncan Cook, Emma Rourke, Ruth Studley, Ian Diamond, Tim Peto, Koen B Pouwels, A Sarah Walker; COVID-19 Infection Survey Team.
   Lancet Reg Health Eur. 2022 Feb:13:100282.
2) https://arxiv.org/abs/2312.17566
   Doublethink: simultaneous Bayesian-frequentist model-averaged hypothesis testing.
   Helen R. Fryer, Nicolas Arning, Daniel J. Wilson

3) https://www.medrxiv.org/content/10.1101/2024.01.01.24300687v1
Identifying direct risk factors in UK Biobank with simultaneous Bayesian-frequentist model-averaged hypothesis testing using Doublethink
Nicolas Arning, Helen R. Fryer, Daniel J. Wilson

4) https://pubmed.ncbi.nlm.nih.gov/36918143/
Understanding how diagnostics influence antimicrobial decision-making is key to successful clinical trial design
Timothy M Rawson, Luke S P Moore
Clin Microbiol Infect. 2023 Jun;29(6):666-669.

5) https://pubmed.ncbi.nlm.nih.gov/38599549/
Distinct patterns of vital sign and inflammatory marker responses in adults with suspected bloodstream infection
Qingze Gu, Jia Wei, Chang Ho Yoon, Kevin Yuan, Nicola Jones, Andrew Brent, Martin Llewelyn, Tim E A Peto, Koen B Pouwels, David W Eyre, A Sarah Walker.
J Infect. 2024 May;88(5):106156.

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3.3 T cells, metabolites and skin disease – multi-omic discovery of new approaches to treatment

Prof. Graham Ogg & Dr. Karmella Naidoo

Contact Email Address:

graham.ogg@ndm.ox.ac.uk;

karmella.naidoo@imm.ox.ac.uk

Project Overview

Human T cell immunology has largely focussed on T cell recognition of peptide antigens presented by HLA molecules. However, it is becoming clear that non-peptide antigens, such as lipids and other metabolites can be presented by HLA-like molecules. CD1a is an HLA class I-like molecule expressed at constitutively high levels by Langerhans cells and presents lipid antigens to T cells. Lipid-reactive T cells account for large populations of skin T cells and have been implicated in contributing to skin inflammation. However, the mechanisms underlying Langerhans cell antigen presentation to CD1a-reactive T cells are not well understood. Here, we aim to investigate Langerhans cell lipid antigen presentation to CD1a-reactive T cells in vitro and in vivo, in order to develop new approaches to treatment. The project will involve analysis of our existing large in-house single cell RNAseq and spatial transcriptomic data of human atopic dermatitis and psoriasis skin disease samples, to identify candidate CD1a pathways for investigation. Next, human Langerhans cells will be isolated from skin and investigated for mechanisms underlying CD1a lipid antigen presentation capacity. This will involve functional CD1a-reactive T cell response analysis, which will include CRISPR gene editing to define underlying mechanisms. The in vitro work will also screen candidate drug molecules for their ability to modulate Langerhans cell CD1a-presentation function. Lastly, pathways and molecules discovered through the computational and in vitro work will be progressed for in vivo investigation using our in-house humanised CD1a transgenic model. The in vivo activities will include spatial transcriptomic analyses at single cell resolution.

The data will generate key biological insights relevant to CD1a mechanisms underlying inflammatory skin disease and will progress translational developments towards patient benefit.

The successful candidate will have opportunities to acquire a deep understanding of human and murine T cell and Langerhans cell functional immunology, combined with multi-omic approaches and the accompanying bioinformatic pipelines. T cell-focussed knowledge and training is of enormous future value in academia or industry career roles where there is increasing interest in manipulating T cells or their cytokines for therapeutic benefit in inflammation and cancer.

Disease Relevance

CD1a-expressing Langerhans cells (LCs) are increased in the skin of patients with atopic dermatitis. In addition, CD1a-restricted T cells have been implicated in the pathogenesis of psoriasis and other skin diseases. Understanding the role of CD1a in both skin inflammatory skin diseases will inform fundamental immunology and will enable us to find more suitable therapeutic targets for the treatment and prevention of disease.

Key Technology

- Flow cytometry – the use of flow cytometry to characterise, immunophenotype cells, cytokines and chemokines either by FACs or Legendplex Assays.
- Spatial Transcriptomics and single cell RNAseq including bioinformatic pipelines
- Imaging Mass Cytometry
- CRISPR gene editing
- In vivo work including inflammatory models and procedures

Training Opportunities

The project will involve extensive training in human and murine immunology with a focus on T cells and antigen presenting cells. This will include core technologies such as flow cytometry, cell culture, functional cell assay, CRISPR gene editing, and will also involve multi-omic approaches including associated bioinformatic pipelines. The project will involve learning about the drug development and commercialisation process. These skills will be of value in broad careers within academia or industry where T cell immunology knowledge is in demand. Specific areas of training certification will include:

- PIL A, and B course which is issued by the HO and enable a Personal License to be obtained in order for in vivo murine experiments to be undertaken.
- Training of Spatial Transcriptomics platforms conducted by the MRC WIMM Advanced Single Cell Omics Facilty (WASCOF).
- Participation in the WIMM graduate student techniques traning programme

Key Publications

1) **Group A Streptococcus induces CD1a-autoreactive T cells and promotes psoriatic inflammation**. Chen Y-L., Ng JSW., Ottakandathil Babu R., Woo J., Nahler J., Hardman CS., Kurupati P., Nussbaum L., Gao F., Dong T., Ladell K., Price DA., Duncan DA., Johnson D., Gileadi U., Koohy H., Ogg GS. 10.1126/sciimmunol.add9232
2) **CD1a promotes systemic manifestations of skin inflammation.** Hardman CS., Chen Y-L., Wegrecki M., Ng SW., Murren R., Mangat D., Silva J-P., Munro R., Chan WY., O'Dowd V., Doyle C., Mori P., Popplewell A., Rossjohn J., Lightwood D., Ogg GS. 10.1038/s41467-022-35071-1
3) **CD1 lipidomes reveal lipid-binding motifs and size-based antigen- display mechanisms.** Huang S, Shahine A, Cheng TY, Chen YL, Ng SW, Balaji GR, Farquhar R, Gras S, Hardman CS, Altman JD, Tahiri N, Minnaard AJ, Ogg GS, Mayfield JA, Rossjohn J, Moody DB. 10.1038/s41590-022-01375-z
4) **CD1a selectively captures endogenous cellular lipids that broadly block T cell response.**Cotton RN., Wegrecki M., Cheng T-Y., Chen Y-L., Veerapen N., Le Nours J., Orgill DP., Pomahac B., Talbot SG., Willis R., Altman JD., de Jong A., Van Rhijn I., Clark RA., Besra GS., Ogg G., Rossjohn J., Moody DB. 10.1084/jem.20202699
5) **CD1a and bound lipids drive T-cell responses in human skin disease**. Ogg G. Rossjohn J., Moody DB 10.1002/eji.202250333

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3.4 Dissecting tissue specific drivers of sexually dimorphic human immune mediated inflammatory diseases

Prof. Chris Buckley, Prof. Mark Coles, Prof. Calli Dendrou & Prof. Kim Midwood

Contact Email Address:

christopher.buckley@kennedy.ox.ac.uk;

mark.coles2@kennedy.ox.ac.uk;

calliope.dendrou@kennedy.ox.ac.uk;

kim.midwood@kennedy.ox.ac.uk

Project Overview

Although many auto-inflammatory (IMID) diseases show sexual dimorphism this differs considerably across disease types with some disease having large differences in both rate and severity tissue pathology (e.g. MS), and other IMIDS showing no dimorphism (e.g. colitis). Rheumatoid arthritis is more common in females, psoriatic arthritis and IMID associated fibrosis more common in males. Although some of this variation results from difference in gene expression in male and female lymphocytes, this does not explain clinical difference observed in tissue specific IMID pathologies. Due to androgen receptor expression on fibroblasts, androgens have the potential to regulate fibroblast – macrophage cross talk in joint synovial tissue, submucosa fibroblasts (intestine) and the dermis of the skin. We previously shown that fibroblasts do so using a combination of secreted signals including sex-specific extracellular matrix patterning that dictates macrophage behaviour. Using a combination of single-cell RNA-seq, multiplex immunohistochemistry, mouse models and in vitro organoids we have found multiple fibroblast subsets in the joint synovium including inflammatory sublining fibroblasts and destructive lining layer fibroblasts and inflammatory fibroblast subsets located in the intestine and dermis. The role of sex and androgen receptor signalling on these pathogenic fibroblast subsets is unknown. Targeting pathological differences in fibroblasts, and their extracellular matrix, might provide a novel method to target human pathology.

Overall Aim:

To compare female and male normal foetal adult,and and patient derived fibroblasts from synovial tissue, gut and skin using state of the art genomics, immunohistochemistry and use function assays to validate the function of these genes in human disease.

Work Plan:

This project aims to understand if there are functionally relevant differences between male and female fibroblasts and how androgen signalling might modulate fibroblast function and interactions with macrophage subsets.

Aim 1:

Utilising a system biology to analysis existing single cell datasets of tissue fibroblasts and macrophages in health and disease from multiple IMIDs tissue biopsies to determine how gene transcription differs between male and female tissue microenvironments.

Aim 2:

Utilise RNAscope and multiplex spatial imaging of tissue samples to validate differences between fibroblasts subsets and extracellular matrix patterning.

Aim 3:

Determine the function of androgen receptor signalling on fibroblasts and fibroblast – macrophage cross talk using an organoids of synovium, gut and skin.

Disease Relevance

Investigating the cellular and molecular differences that underpin the distinct aetiology and progression of male and female disease is critical to better understand differences in treatment response to existing therapies and to enable the most effective diagnosis and treatment of IMIDs, fibrosis and cancer. Mechanistically underexplored, the role of tissue specific drivers of sexually dimorphic immune disease also holds the key to the development of new strategies to treat human disease.

Key Technology

During the project key skills will be developed including systems approaches to analysing single-cell and spatially-resolved omics datasets, this will involve familiarisation with basic python and R.

The project involves multiplex imaging, preparing and staining tissue samples, staining protocols and image analysis. Skills in tissue culture, development of tissue organoids, processing primary human blood and tissues, and analysing outputs using qPCR and flow cytometry will also be developed.

Training Opportunities

The Kennedy Institute is a world-renowned research centre, located in the heart of the University of Oxford's Old Road campus sitting alongside the COI, housing fundamental and clinician scientists working on diverse aspects of immunology and inflammation. Students will join a vibrant postgraduate community at the Kennedy. The PhD programme includes a core curriculum of lectures in the first term of year 1, students will take part in group meetings from each of the labs of the supervisory team, and have the opportunity to attend seminars from world leading scientists both in the department and across the wider University. Students will present their work at national and international meetings.

Key Publications

1) A life-course approach to women's health. Nat Med 30, 1 (2024). https://doi.org/10.1038/s41591-023-02777-8
2) Buckley, C.D., Midwood, K.S. Tracing the origins of lung fibrosis. Nat Immunol 25, 1517–1519 (2024). https://doi.org/10.1038/s41590-024-01934-6
3) Fibroblasts as immune regulators in infection, inflammation and cancer.Davidson S, Coles M, Thomas T, Kollias G, Ludewig B, Turley S, Brenner M, Buckley CD.Nat Rev Immunol. 2021 Nov;21(11):704-717. doi: 10.1038/s41577-021-00540-z. Epub 2021 Apr 28.PMID: 33911232
4) Korsunsky I, Wei K, Pohin M, Kim EY, Barone F, Major T, Taylor E, Ravindran R, Kemble S, Watts GFM, Jonsson AH, Jeong Y, Athar H, Windell D, Kang JB, Friedrich M, Turner J, Nayar S, Fisher BA, Raza K, Marshall JL, Croft AP, Tamura T, Sholl LM, Vivero M, Rosas IO, Bowman SJ, Coles M, Frei AP, Lassen K, Filer A, Powrie F, Buckley CD, Brenner MB, Raychaudhuri S. Cross-tissue, single-cell stromal atlas identifies shared pathological fibroblast phenotypes in four chronic inflammatory diseases, Med (N Y) 2022 Jul 8;3(7):481-518.e14. doi: 10.1016/j.medj.2022.05.002

5) Curion F, Rich-Griffin C, Agarwal D, Ouologuem S, Thomas T, Theis FJ, Dendrou CA. (2023) Panpipes a pipeline for multiomic single-cell and spatial transcriptomic data analysis. bioRxiv, doi:10.1101/2023.3.11.532085.
6) Thomas T, Rich-Griffin C, Pohin M, Friedrich M et al. (2023) A longitudinal single-cell therapeutic atlas of anti-tumour necrosis factor treatment in inflammatory bowel disease. bioRxiv, doi:10.1101/2023.05.05.539635.

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3.5 Immune and epigenetic consequences of airways infections

Prof. Timothy Hinks & Prof. Paul Klenerman

Contact Email Address:

timothy.hinks@ndm.ox.ac.uk;

paul.klenerman@medawar.ox.ac.uk

Project Overview

Asthma is the world's most common chronic lung disease and arises from the complex interplay of early life exposures including bacterial and viral respiratory infections such as Haemophilus influenzae and respiratory syncytial virus (RSV). How these early life infections cause asthma is unknown. We believe it is through persistent epigenetic changes particularly in the airway epithelium.

Aim: to discover the immune and epigenetic mechanisms by which these early infections drive development of chronic airways inflammation.

Our group are using a number of approaches which include murine, human and in vitro studies. We have developed a unique, novel murine model of persistent bacterial infection as well as using acute viral infections. We explore the immunology of host-pathogen interactions in vivo, including effects on T cell responses, macrophages and epithelial cells. We also use primary human airway tissue samples from research bronchoscopies, as well as induced pluripotent stem cell derived airway epithelial cells, and samples from collaborators with paediatric (Andrew Pollard, Oxford) and adult (Peter Bradding, Leicester) cohorts. We collaborate with several groups across Oxford including Julian Knight (Genomics), Sally Cowley (iPSC).

We apply a number of techniques which in our lab typically includes classical cellular immunology, spectral cytometry (Cytek Aurora), single cell RNAseq (10X), CITEseq, bulk or single cell ATACseq, and spatial transcriptomic analyses. The research questions arise from recent clinical observations and are highly clinically relevant with potential implications for the world's most common lung diseases.

Disease Relevance

- Asthma affects 1 in 12 people in UK and 350 million people worldwide. It affects people from early childhood to old age and is a major cause of time lost from work or school, and is responsible for >1% of disability adjusted life years globally. Its prevalence is rising rapidly, especially in countries in East Asia including China.
- This project is working ultimately to a cure for some forms of asthma within the next few decades. As a clinician scientist Prof Hinks provides specialist care to those with severe asthma from a population of 2.5 million people, and the questions his group addresses arise from his work with these patients.
- The project is identifying the specific molecular mechanisms of this disease with direct line of sight to curative approaches which will take us beyond the current therapies which require long term, often lifelong daily treatment.

Key Technology

We apply a number of techniques which in our lab typically include:

- Classical cellular immunology
- Spectral cytometry (Cytek Aurora)
- single cell RNAseq (10X), CITEseq
- DNA methylation analysis*
- Bulk or single cell ATACseq*
- Spatial transcriptomic analyses (10X Xenium)
- Bioinformatic analyses of these datasets supported by our group's senior bioinformatician*

* This project will primarily depend on these techniques
Work will include murine and human samples

Training Opportunities

- The student will be trained in murine handling and experimentation according to strict UK Home Office welfare standards
- The student will be trainined in cellular immunology, flow cytometry, single cell RNA and ATAC sequencing; library prep and sequencing analysis.
- The student will be trained in bioinformatic analyses of the datasets generated

Prospective students are encouraged to contact Prof Hinks directly to discuss specific details of this project to find out more about the precise research questions which most interest them.

Key Publications

1) Jabeen MF, Sanderson ND, Tinè M, Donachie G, Barber C, Azim A, Lau LCK, Brown T, Pavord ID, Chauhan A, Klenerman P, Street TL, Marchi E, Howarth PH, Hinks TSC. Species-level, metagenomic and proteomic analysis of microbe-immune interactions in severe asthma. *Allergy* 2024 Aug 11. https://doi.org/10.1111/all.16269
2) Howell I, Yang F, Brown V, Cane J, Marchi E, Azim A, Busby J, McDowell PJ, Diver SE, Borg C, Heaney LG, Pavord ID, Brightling CE, Chaudhuri R, Hinks TSC. Airway proteomics reveals broad residual anti-inflammatory effects of prednisolone in mepolizumab-treated asthma. *J Allergy Clin Immunol.* 2024 Aug 2:S0091-6749(24)00777-2. https://doi.org/10.1016/j.jaci.2024.07.020
3) Hinks TSC, Marchi E, Jabeen M, Olshansky M, Kurioka A, Pediongco TJ, Meehan BS, 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 Tissue Repair Functionality *Cell Rep.* 2019 Sep 17;28(12):3249-3262.e5.
4) Wang H, D'souza C, Lim XY, Kostenko L, Pediongco TJ, Eckle SBG, Meehan BS, Shi M, Wang N, Li S, Liu L, Mak JYW, Fairlie Dp, Iwakura Y, Gunnersen JM, Stent AW, Godfrey DI, Rossjohn J, Westall GP, Kjer-Nielsen L, Strugnell RA, Mccluskey J, Corbett AJ, Hinks TSC, Chen Z. 2018. MAIT cells protect against pulmonary *Legionella longbeachae* infection. *Nat Commun*, **9** (1), pp. 3350. https://www.nature.com/articles/s41467-018-05202-8

5) Van Wilgenburg B, Loh L, Chen Z, Pediongco TJ, Wang H, Shi M, Zhao Z, Koutsakos M, Nüssing S, Sant S, Wang Z, D'souza C, Jia X, Almeida CF, Kostenko L, Eckle SBG, Meehan BS, Kallies A, Godfrey DI, Reading PC, Corbett AJ, Mccluskey J, Klenerman P, Kedzierska K, Hinks TSC. 2018. MAIT cells contribute to protection against lethal influenza infection in vivo. *Nat Commun*, **9** (1), pp. 4706 https://www.nature.com/articles/s41467-018-07207-9

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3.6 Detecting and dissecting host-pathogen genetic interactions

Dr. Azim Ansari & Prof. Paul Klenerman

Contact Email Address:

azim.ansari@ndm.ox.ac.uk;

paul.klenerman@ndm.ox.ac.uk

Project Overview

Dive into the dynamic world of infectious diseases, where the intricate interplay between host and pathogen genetics shapes the outcome of infections. Our research leverages **paired host and pathogen genomic data** to uncover the genetic underpinnings of disease phenotypes. We have generated paired host and pathogen genomic data from the same individuals in large cohorts of HCV, HBV, SAR-CoV-2 and dengue infections. This is your chance to model and analyse these data and be at the forefront of ground-breaking discoveries that could transform our understanding of these infections.

Our Mission:

We are a dynamic, interdisciplinary team dedicated to understanding the intricate interplay between host and pathogen genetics in globally important infections. Moving beyond traditional approaches that study host and pathogen genetics in isolation, we generate and analyse host and pathogen genomic data from the same individuals which allows us to detect how host-pathogen genomic interactions shape disease outcomes.

Our Approach:

Leveraging cutting-edge sequencing technologies and advanced computational methods, we analyse paired host and pathogen genomic data from well-characterized cohorts infected with globally significant pathogens such as HBV, HCV, HIV and dengue. This unique approach allows us to:

- Uncover hidden links: Identify host polymorphisms associated with specific pathogen variations, revealing potential targets for intervention.
- Expose evolutionary arms races: Pinpoint pathogen sites under strong host genetic selection, providing insights into mechanisms of pathogen adaptation and immune evasion.
- Decipher the genetic architecture of disease: Disentangle the individual and interactive effects of host and pathogen genetic factors on disease phenotypes.

Project Highlights:

- Interdisciplinary Collaboration: Work alongside leading clinicians, immunologists, and scientists from around the world, including partners in Pakistan, Thailand, Vietnam and China.
- Tailored Research Opportunities: Projects are flexible and can be customized to align with your interests. Whether you're passionate about bioinformatics, evolution, or computational statistics, there's a place for you here.
- Real-World Applications: Your research could lead to breakthroughs in our understanding and improving patient stratification and treatment efficacy in high-burden infections.

Potential Project Areas:

As a PhD student in our group, you will join a vibrant, collaborative environment at the forefront of infectious diseases. We offer a range of exciting project areas, including:

- Unravelling the genetic basis of host-pathogen interactions: Investigating the impact of host genetic variation on pathogen diversity and how the interaction between the two impacts clinical outcomes of infections such as HCV, HBV and dengue.
- Investigating the antigen presentation pathway: Investigating the role of genetic variation in antigen presentation pathway and the HLA region in shaping infection outcomes.
- Understanding Sex differences in infectious diseases: Generating a better understanding of how sex differences impact outcomes of infections and the contribution of genetics.
- Developing novel statistical methodologies: Pioneering new analytical approaches to integrate and interrogate paired host-pathogen genomic data.

We are particularly interested in students passionate about:

- Infectious diseases
- Evolution
- Bioinformatics
- Host-pathogen interactions
- Population genetics
- Computational statistics
- Machine learning

We are flexible and open to tailoring projects to your specific interests. We also encourage you to propose your own research ideas within our scope of expertise.

Join Our Team:

We are a highly collaborative group with strong ties to clinicians, immunologists, and wet-lab scientists both nationally and internationally. This provides our students with unique opportunities for interdisciplinary collaboration and exposure to diverse research perspectives.

Disease Relevance

By understanding the genetic underpinnings of host-pathogen interactions, our research has the potential to:

- *Revolutionize our understanding of infectious disease pathogenesis:* Providing fundamental insights into human biology and the dynamics of host-pathogen co-evolution.
- *Drive the development of novel therapeutics:* Contribute to the development of more effective vaccines, drug targets, and novel immunotherapies by understanding how genetic variations drive pathogen adaptations and disease heterogeneity.
- *Enable personalized medicine:* Facilitating patient stratification for optimized treatment strategies based on individual genetic profiles.

Key Technology

We have expertise in Statistical Genetics, Evolution, Bioinformatics, Machine Learning, Immunology, Pathogen Genome Sequencing, Human Genetics and a focus on Chronic viral infections of HCV and HBV.

Training Opportunities

Depending on the project, you will gain expertise in Statistics, Machine Learning and Computational Biology.

Key Publications

1) Ansari M., Pedergnana V., L C Ip C. *et al.* Genome-to-genome analysis highlights the effect of the human innate and adaptive immune systems on the hepatitis C virus. *Nat Genet* **49**, 666–673 (2017). https://doi.org/10.1038/ng.3835
2) Ansari MA, Aranday-Cortes E, L C Ip C, et al. Interferon lambda 4 impacts the genetic diversity of hepatitis C virus. Elife. 2019 Sep 3;8:e42463. doi: 10.7554/eLife.42463
3) Smith, D.A., Fernandez-Antunez, C., Magri, A. *et al.* Viral genome wide association study identifies novel hepatitis C virus polymorphisms associated with sofosbuvir treatment failure. *Nat Commun* **12**, 6105 (2021). https://doi.org/10.1038/s41467-021-25649-6
4) Simmonds P, Ansari MA. Extensive C->U transition biases in the genomes of a wide range of mammalian RNA viruses; potential associations with transcriptional mutations, damage- or host-mediated editing of viral RNA. PLoS Pathog. 2021 Jun 1;17(6):e1009596. doi: 10.1371/journal.ppat.1009596
5) Ansari MA, Didelot X. Bayesian Inference of the Evolution of a Phenotype Distribution on a Phylogenetic Tree. Genetics. 2016 Sep;204(1):89-98. doi: 10.1534/genetics.116.190496. Epub 2016 Jul 13. PMID: 27412711; PMCID: PMC5012407

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3.7 Optimising surveillance and treatment of infectious diseases using AI and Big Data

Prof. David Eyre & Prof A. Sarah Walker

Contact Email Address:

david.eyre@bdi.ox.ac.uk;

sarah.walker@ndm.ox.ac.uk

Project Overview

Infections pose a major risk to health globally, and also in the UK where they are responsible for 7% of deaths, 21% of all workdays lost, and cost £30bn a year. Antimicrobial resistance (AMR) threatens effective treatment of infection and healthcare associated infections (HCAIs) impact 1 million people each year in the UK.

Advances in data availability and new artificial intelligence (AI) methods offer the chance to develop:

- **More responsive, comprehensive, and automated HCAI/AMR surveillance** generating better breadth and depth of intelligence to drive action and changes in practice to protect diverse populations at local, regional, and national levels.
- **Predictive tools to improve care of individual patients** and combat AMR.

Methods, infrastructure and skills to optimally use rapidly-evolving electronic healthcare record and patient-contributed data, and emerging AI technologies.

Several possible specific projects are available, including:

- Developing/testing automated electronic surveillance approaches for rapidly detecting changes in infections and identifying at-risk populations; and deploying these tools in hospitals and national systems
- Extending and piloting in the NHS predictions of personal AMR risk to optimise infection treatment, prevention and control, developing generalisable methods that can update over time/to new locations, and approaches for safely implementing them
- Pre-emptive surveillance, investigating which metrics of hospital processes (e.g. isolation/screening/diagnostic use/cleaning) are associated with HCAI/AMR to inform prevention

Related projects can also be developed, please contact david.eyre@bdi.ox.ac.uk.

Large-scale comprehensive healthcare data are available, including a highly-detailed clinical data from 1% of the UK population spanning over 10 years. There will also be opportunities for working with national data and combined community/hospital data from whole UK regions.

Disease Relevance

The proposed projects are directly relevant to the surveillance, management, treatment and prevention of infections. Supervisors include Consultants in Infection/Microbiology at the Oxford University Hospitals NHS Foundation Trust, ensuring that the project is tightly linked to real-world clinical questions and practice.

Key Technology

- Linked and de-identified electronic health records
- Statistical and epidemiological analysis
- Machine learning

Training Opportunities

The candidate will learn a wide range of state-of-the-art statistical and machine learning approaches, based in the Big Data Institute within a broad infection research consortium.

The DPhil will involve working closely with the UK's national public health agency, UKHSA, as well as NHS hospitals, clinicians and other healthcare providers. Industry partnerships to further develop successful tools and approaches will be supported by close working with University's technology transfer organization – Oxford University Innovation.

The ideal candidate would like solving real-world applied problems that matter and are also technically exciting and challenging. They should have excellent computational and numerical skills (although competence in specific techniques or healthcare experience is not a pre-requisite). Someone who also feels at home in a welcoming, enthusiastic and multidisciplinary team will thrive in this DPhil.

Key Publications

1) Gu Q, Wei J, Yoon CH, …, Eyre DW*, Walker AS*. 2024. Distinct patterns of vital sign and inflammatory marker responses in adults with suspected bloodstream infection. J Infect 88:106156
2) Wei J, Uppal A, Nganjimi C, …, Eyre DW. 2024. No evidence of difference in mortality with amoxicillin versus co-amoxiclav for hospital treatment of community-acquired pneumonia. J Infect 88:106161
3) Eyre DW, Futschik M, Tunkel S, et al. 2023. Performance of antigen lateral flow devices in the UK during the alpha, delta, and omicron waves of the SARS-CoV-2 pandemic: a diagnostic and observational study. Lancet Inf Dis 23:922-932.
4) Eyre DW, Taylor D, Purver M, et al. 2022. Effect of Covid-19 Vaccination on Transmission of Alpha and Delta Variants. New Eng J Med 386: 744-756.
5) Soltan AS, Yang J, Pattanshetty R, …, Eyre DW, Clifton DA. 2021. Real-world evaluation of AI-driven COVID-19 triage for emergency admissions: External validation & operational assessment of lab-free and high-throughput screening solutions. Lancet Digital Health 4: e266-278.

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3.8 Development of murine and iPSC-derived organoid models for T cell mediated killing of virus infected cells

Dr. Jack Tan & Dr. Peter Wing

Contact Email Address:

jack.tan@rdm.ox.ac.uk;

peter.wing@ndm.ox.ac.uk

Project Overview

The development of advanced in vitro models has revolutionised our ability to study complex biological processes in controlled environments. This project introduces a novel approach to investigating respiratory viral infections by combining induced pluripotent stem cell (iPSC)-derived pneumocyte organoids with high-containment confocal imaging techniques. By establishing a tractable mouse organoid model, we aim to elucidate the intricate dynamics of infection and the crucial interplay between immune cells, particularly CD8+ T cells, and infected respiratory tissues. This innovative platform will enable real-time monitoring of CD8+ T cell-mediated killing, providing unprecedented insights into the regulation of antiviral immune responses in a physiologically relevant context.

Initially proof of principle experiments will be designed to grow and optimise the culture conditions of mouse lung and spleen co-culture organoids. The composition of the lung and spleen organoids will be determined using FACS. These cultures will then be stimulated with an antigen of interest (e.g. influenza virus, SARS-CoV-2 virus and the natural mouse pathogen, mouse hepatitis virus) and the immune cell composition will be determined and compared to unstimulated controls. These studies will optimise the culture conditions and infection dynamics for subsequent co-culture studies.

We next will study the interplay between the lung and spleen co-culture organoids. For this, we will use influenza as a disease model. Lung and spleen co-culture organoids will be prepared from mice pre-infected and recovered from a sublethal influenza infection. The lung organoids will be stimulated with a homologous or heterologous influenza virus and the activation of B cell and T cell of the spleen culture will be assessed. Killing of infected lung cells by T cells will be quantified as well as antigen specific (NP) CD8 T cells using tetramer staining post stimulation, compared to unstimulated control. Anti-influenza antibody response in the culture supernatant post stimulation will also be measured using in vitro immunoassays (e.g. ELISA and microneutralisation assays).

Infected organoids will be co-cultured with SARS-CoV-2-specific CD8+ T cell clones and T cell lines isolated from the donors with history of SARS-CoV-2 infection or vaccination. The killing of infected cells by the co-cultured SARS-CoV-2 specific T cells will be assessed using previously established methodology and the use of the fluorescent mNeonGreen-SARS-CoV-2 reporter virus will enable direct and easy quantification of virus infected cells. Further using the Zeiss CD7 live cell confocal microscope within the OPIC CL3 facility we will assess real-time measurement of T-cell killing of SARS-CoV-2 infected cells in a physiologically relevant organoid model and subsequent assessment of the factors contributing to viral control or immunopathology. Anti-SARS-CoV-2 antibody response in the culture supernatant following stimulation will be quantified utilising in vitro immunoassays (e.g. ELISA and microneutralisation assays).

This work will establish a tractable mouse organoid model to assess infection dynamics and the interplay between immune cell regulation of respiratory viral infections. Through using an iPSC derived pneumocyte organoid model combined with high-containment confocal imaging we will characterise the dynamics of CD8 T cell killing in a live cell setting with real time monitoring.

Disease Relevance

This project develops an advanced in vitro model for investigating respiratory viral infections utilizing induced pluripotent stem cell (iPSC)-derived pneumocyte organoids and high-containment confocal imaging. The key aspects of this research include:

- Enhancing the understanding of infection dynamics and immune responses
- Examining immune cell interactions, with a particular focus on CD8+ T cells
- Conducting real-time monitoring of antiviral immune responses
- Optimising co-culture conditions for mouse lung and spleen organoids
- Investigating cross-reactive immunity to various influenza strains
- Conducting SARS-CoV-2-specific research utilising T cell clones and fluorescent reporter viruses
- Exploring potential applications in therapeutic and vaccine development
- Advancing organoid technology
- Reducing reliance on animal testing (3R)
- Broadening implications for immunology research

The model aims to provide comprehensive insights into viral infections and immune responses.

Key Technology

The development of advanced in vitro models has revolutionized our ability to study complex biological processes in controlled environments. The key technology in this project involves:

- iPSC-derived pneumocyte organoids: Advanced in vitro model of lung tissue.
- High-containment confocal imaging: Enables real-time monitoring of viral infections and immune responses.
- Co-culture systems: Optimized conditions for combining lung and spleen organoids.
- Fluorescent reporter viruses: Used for visualizing viral infection dynamics.
- T cell clones: Specifically for SARS-CoV-2 research.

This integrated approach allows for detailed study of respiratory viral infections, immune cell interactions, and antiviral responses in a controlled, human-relevant system. The technology aims to reduce animal testing while providing insights into infection mechanisms and potential therapeutic strategies.

Training Opportunities

This project offers several advanced training opportunities in respiratory viral infection research techniques:

1) iPSC-derived pneumocyte organoid culture:
   - Development and optimisation of mouse lung and spleen organoid culture conditions
   - Implementation of co-culturing techniques for lung and spleen organoids
2) Flow cytometry (FACS):
   - Analysis of organoid composition
   - Identification of immune cell populations
3) Viral infection models:
   - Utilization of influenza virus, SARS-CoV-2, and mouse hepatitis virus
   - Management and cultivation of infected organoids
4) Immunological techniques:
   - Execution of T cell activation assays
   - Assessment of B cell responses
   - Performance of tetramer staining for antigen-specific CD8 T cells
   - Conduct of ELISA and microneutralisation assays for antibody responses
5) High-containment confocal imaging:
   - Operation of Zeiss CD7 live cell confocal microscope in CL3 facility
   - Real-time imaging of T cell-mediated killing
6) Fluorescent reporter virus techniques:
   - Application of mNeonGreen-SARS-CoV-2 reporter virus
   - Quantification of virus-infected cells
7) T cell isolation and culture:
   - Isolation of SARS-CoV-2-specific CD8+ T cell clones and lines
   - Cultivation of T cells with infected organoids
8) Data analysis and interpretation:
   - Evaluation of infection dynamics
   - Examination of immune cell regulation of viral infections
   - Assessment of contributing factors

Key Publications

1) Lee, Jeffrey Y., Peter A. C. Wing, Dalia S. Gala, Marko Noerenberg, Aino I. Jarvelin, Joshua Titlow, Xiaodong Zhuang, et al. 2022. 'Absolute Quantitation of Individual SARS-CoV-2 RNA Molecules Provides a New Paradigm for Infection Dynamics and Variant Differences.' *eLife* 11 (January):e74153. https://doi.org/10.7554/eLife.74153.
2) Wing, Peter A. C., Thomas P. Keeley, Xiaodong Zhuang, Jeffrey Y. Lee, Maria Prange-Barczynska, Senko Tsukuda, Sophie B. Morgan, et al. 2021. 'Hypoxic and Pharmacological Activation of HIF Inhibits SARS-CoV-2 Infection of Lung Epithelial Cells.' *Cell Reports* 35 (3): 109020. https://doi.org/10.1016/j.celrep.2021.109020.

3) Wing, Peter A. C., Maria Prange-Barczynska, Amy Cross, Stefania Crotta, Claudia Orbegozo Rubio, Xiaotong Cheng, James M. Harris, et al. 2022. 'Hypoxia Inducible Factors Regulate Infectious SARS-CoV-2, Epithelial Damage and Respiratory Symptoms in a Hamster COVID-19 Model.' *PLoS Pathogens* 18 (9): e1010807. https://doi.org/10.1371/journal.ppat.1010807.

4) Yang, Hongbing, Hong Sun, Simon Brackenridge, Xiaodong Zhuang, Peter A. C. Wing, Max Quastel, Lucy Walters, et al. 2023. 'HLA-E-Restricted SARS-CoV-2-Specific T Cells from Convalescent COVID-19 Patients Suppress Virus Replication despite HLA Class Ia down-Regulation.' *Science Immunology* 8 (84): eabl8881. https://doi.org/10.1126/sciimmunol.abl8881.

5) Ming Z.M. Zheng, Tiong Kit Tan, Fernando Villalon-Letelier, Hilda Lau, Yi-Mo Deng, Svenja Fritzlar, Sophie Valkenburg, Haogao Gu, Leo Poon, Patrick C. Reading, Alain R. Townsend, Linda M. Wakim. "Single-cycle influenza virus vaccine generates lung $CD8^+$ Trm that cross react against viral variants and subvert the emergence of virus escape mutants" *Science Advances 2023. DOI: 10.1126/sciadv.adg3469*

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3.9 Evasion of innate immunity by monkeypox virus

Dr. Geoffrey L Smith & Dr. Yiqi Zhao

Contact Email Address:

geoffrey.smith@path.ox.ac.uk;

yiqi.zhao@path.ox.ac.uk

Project Overview

This project will identify host proteins that restrict the replication of orthopoxviruses such as vaccinia virus and monkeypox virus and study their mechanisms of action. Do they function in innate immunity to promote an antiviral response? Or do they target and modify specific virus proteins to antagonize their function? The project will also investigate virus countermeasures that antagonize these host factors and enable the viruses to evade or suppress the host response to infection. This basic science project will provide fundamental information about how cells respond to poxvirus infection and how these viruses may escape control and cause disease. The project is based on proteomic studies of cells infected with these viruses that showed that hundreds of cellular proteins are reduced in abundance after infection, mostly by proteolytic degradation. In the cases of 3 such proteins, HDAC4, HDAC5 and TRIM5α, it has been demonstrated that these have anti-viral activity and are antagonized by viral counter-measures. Further, a detailed mechanistic study of TRIM5α and virus countermeasures led to the proposed re-purposing of existing drugs against monkeypox virus.

Disease Relevance

In 2022 monkeypox virus (MPXV) emerged from its natural reservoir in Africa to cause a global epidemic with more that 100,000 human cases and > 200 deaths. This epidemic was caused by clade IIb MPXV strains that have a low case fatality rate (cfr, 0.2%). In 2023, another MPXV epidemic started in central Africa (Democratic Republic of the Congo, DRC) caused by clade Ib MPXVs. These clade I viruses are more dangerous in humans and have a cfr of 2-10%. Clade Ib viruses have now spread beyond DRC, to other African nations and outside Africa consequently the WHO declared a Public Health Emergency of International Concern in August 2024. To prevent or treat the disease mpox (caused by MPXV), additional vaccines and drugs are needed. The lead anti-poxvirus drug, tecovirimat, recently was shown to have little efficacy against mpox in DRC and drug-resistant MPXV strains have emerged. Therefore, new anti-poxviral drugs are needed. Understanding immune evasion strategies of MPXV strains and differences between the clade I and clade II viruses that influence different disease outcomes will contribute to development of additional thereapies against these viruses.

Key Technology

- Proteomics by mass spectrometry
- Interactomics: idenitification of virus protein binding partners in cells
- Growth and purification of viruses
- Expression and purification of proteins from eukaryotic cells or E. coli
- CRISPR-cas9 genome editing
- Structural determination of proteins alone and in complex

Training Opportunities

The project will provide a broad training in molecular biology, virology, innate immunity, structural biology and virus-host interactions. It is particulrly suitable for a PhD student with expertise in biological science, although medical students might also be suitable. The student will be trained in a broad range of tecnhiques that are applicable to many areas of biological or medical science, using cutting-edge technology and state of the art equipment. The student will be based in the Dunn School of Pathology and have collabortors within COI for structural biology and proteomics.

Key Publications

1) Albarnaz, J.D., Ren, H., Torres, A.A., Shmeleva, E.V., de Melo, C.M.A.G., Bannister, A.J. & Smith, G.L. (2022). Molecular mimicry of NF-kappaB by vaccinia virus protein enables selective inhibition of antiviral responses. *Nature Microbiol.* 7, 154-68
2) Talbot-Cooper, C., Pantelejevs, T., Shannon, J.P., Cherry, C.R., Au, M.T., Hyvönen, M., Hickman, H.D. & Smith, G.L. (2022). Poxviruses and paramyxoviruses use a conserved mechanism of STAT1 antagonism to inhibit interferon signaling. *Cell Host Microbe* 30, 1-16
3) Shmeleva, E.V., Gomez de Agüero, M., Wagner, J., Enright, A.J., Macpherson, A.J., *Ferguson, B.J., & *Smith, G.L. (2022) Smallpox vaccination induces a substantial increase in commensal skin bacteria that promote pathology and influence the host response. *PLoS Pathogens,* 18, e1009854
4) Zhao, Y., Lu, Y., Richardson, S., Sreekumar, M., Albarnaz, J.D. & Smith, G.L. (2023). TRIM5α restricts poxviruses and is antagonized by CypA and the viral protein C6. *Nature*, 620, 873-880
5) Lu, Y., Zhao, Y., Gao, C., Suresh, S., Men, J., Sawyers, A., & Smith, G.L. (2024). HDAC5 is a positive regulator of IRF3 activation and is targeted for degradation by protein C6 from orthopoxviruses including monkeypox virus and variola virus. *Cell Reports* 43, 113788:doi: 10.1016/j.celrep.2024.113788

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3.10 Nucleic Acid Sensing During Virus Infection

Prof. Jan Rehwinkel & Dr. Adán Pinto Fernández

Contact Email Address:

jan.rehwinkel@imm.ox.ac.uk

adan.pintofernandez@ndm.ox.ac.uk

Project Overview

Virus infection is a constant threat to the cells of all living organisms. To counter this threat, cellular receptors detect virus presence and activate potent antiviral immune responses. Some of these sensors of virus presence signal for the activation of innate immune genes, which in humans include type I interferons. These cytokines then alert neighbouring, uninfected cells and induce the expression of hundreds of genes, many of which encode proteins with direct antiviral function. Viruses in turn have developed strategies to counteract and evade detection and control by the innate immune system. As such, cells and viruses are in a dynamic arms race in which host defence mechanisms and viral counter-measures rapidly co-evolve. Our aim is to investigate the molecular mechanisms by which mammalian cells recognise and respond to infection by viruses.

Viruses cannot replicate and complete their life cycles without introducing their RNA or DNA genomes into host cells. Nucleic acid sensing is therefore a broadly effective cellular defence strategy for the detection of virus infection. Nucleic acid sensors engage different signalling pathways to induce an antiviral state. This includes the production of interferons and other cytokines, stress responses and programmed cell death.

The presence of vast quantities of cellular RNAs and DNAs in healthy, uninfected cells necessitates molecular mechanisms of self / non-self discrimination and poses the risk of unwanted immune responses in the absence of infection. Indeed, nucleic acid sensing pathways have been linked to autoinflammatory and autoimmune diseases. Moreover, nucleic acids are also involved in priming immune responses targeting cancers and are potent adjuvants for vaccination. The study of nucleic acid sensing is thus important to our understanding of host-pathogen interactions and the aetiology of some autoimmune diseases, and is likely to inform the development of novel therapies.

Our research focuses on the molecular biology of activation and regulation of innate immune receptors that survey the cytosol. We use a variety of virus infection models including influenza A virus, HIV and other retroviruses, flaviviruses such as Zika virus, herpesviruses and SARS-CoV-2. In addition, we are studying the role of nucleic acid sensing in inflammatory diseases and in cancer. We are particularly interested in RIG-I-like receptors and cytosolic DNA receptors such as cGAS. Furthermore, we are interested in SAMHD1, which restricts virus infection and is also linked to Aicardi-Goutières syndrome – an autoinflammatory disease driven by interferons – and cancer. Some of the projects in our lab look at:

- How cGAMP is packaged into viral particles to trigger antiviral immunity upon infection, and if this can be used to enhance vaccine responses.
- How unusual DNA and RNA molecules in the Z-conformation, a possible by-product of viral infection, are sensed by ZBP1 and other proteins in the innate immune system.
- The mechanisms by which the Zika Virus, Varicella Zoster Virus, HSV-1 and SARS-CoV-2 are detected by the innate immune system, and how these viruses counteract detection.

Jan Rehwinkel would be delighted to discuss these and related projects further with interested applicants (jan.rehwinkel@imm.ox.ac.uk).

Disease Relevance

Viral infection, autoimmunity, cancer

Key Technology

- Molecular biology
- Proteomics

Training Opportunities

Based in the MRC Translational Immune Discovery Unit at the Weatherall Institute of Molecular Medicine, with access to state-of-the-art facilities, we provide an opportunity for training in a broad range of different techniques, including cell culture, molecular biology, immunology, proteomics, lipidomics, virology and mouse models. Our work additionally benefits from close collaboration with many scientists. The successful candidate will be supervised by Jan Rehwinkel (who recently won the Andrew McMichael Medal for excellent graduate supervision) at weekly 1-to-1 meetings and co-supervised by Adán Pinto-Fernández, a Career Development Fellow at the COI institute at the Nuffield Department of Medicine. Additional day-to-day supervision will be provided by an experienced member of the Rehwinkel lab. The successful candidate will also present on a weekly basis at laboratory meetings and will expand their knowledge of the field through a regular journal club. Jan Rehwinkel is highly supportive of students' career development and encourages students to attend and participate in scientific conferences.

Key Publications

1) Hertzog J, Zhou W, Fowler G, Rigby RE, Bridgeman A, Blest HTW, Cursi C, Chauveau L, Davenne T, Warner BE, Kinchington PR, Kranzusch PJ, Rehwinkel J. Varicella-Zoster Virus ORF9 Is an Antagonist of the DNA Sensor cGAS. The EMBO Journal. 2022; doi: 10.15252/embj.2021109217.
2) Tang Q, Rigby RE, Young GR, Hvidt AK, Davis T, Tan TK, Bridgeman A, Townsend AR, Kassiotis G, Rehwinkel J. Adenosine-to-inosine editing of endogenous Z-form RNA by the deaminase ADAR1 prevents spontaneous MAVS-dependent type I interferon responses. Immunity. 2021; 54(9):1961-1975.e5.
3) Sampaio NG, Chauveau L, Hertzog J, Bridgeman A, Fowler G, Moonen JP, Dupont M, Russell RA, Noerenberg M, Rehwinkel J. The RNA sensor MDA5 detects SARS-CoV-2 infection. Sci Rep. 2021; 11(1):13638.
4) Maelfait J, Liverpool L, Bridgeman A, Ragan KB, Upton JW, Rehwinkel J. Sensing of viral and endogenous RNA by ZBP1/DAI induces necroptosis. The EMBO Journal. 2017; 36(5):604-616.
5) Bridgeman A, Maelfait J, Davenne T, Partridge T, Peng Y, Mayer A, Dong T, Kaever V, Borrow P, Rehwinkel J. Viruses transfer the antiviral second messenger cGAMP between cells. Science. 2015; 349:1228-1232.

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3.11 Spatial exploration of hypoxic signalling and inflammation in hepatitis B associated liver cancer

Prof. Jane McKeating & Prof. Tao Dong

Contact Email Address:

jane.mckeating@ndm.ox.ac.uk;

tao.dong@ndm.ox.ac.uk

Project Overview

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

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

Disease Relevance

Hepatitis B virus (HBV) is a global health challenge with over 290 million infections and major cause of liver disease and cancer. Hepatocellular carcinoma (HCC) is one of the fastest rising and most common causes of cancer-related deaths in the world and there is an urgent need for new therapies.

Key Technology

- Digital spatial transcriptomics and immune profiling of human tissue samples from patients with diagnosed HCC.
- Nearest neighbour analysis using MuSpan pipeline to examine cellular interactions at the micro-anatomic level.

Training Opportunities

The student will join a dynamic and lively team of biologists in the McKeating and Dong laboratories that bring complementary expertise in hypoxic signalling and T cell immunology. Importantly this project is supported by a long-standing collaboration with clinical hepatologists Emma Culver and Alberto Quaglia. This interdisciplinary project will provide a unique training environment to gain expertise in the biology of hepatocellular carcinoma and state-of-art training in digital spatial profiling and bio-informatic analysis of single cell transcriptomic data sets. Transferable skills include oral presentations at joint lab meetings, critical review of published scientific literature by contributing to journal clubs and scientific writing by reviewing and drafting manuscripts for publication. The student will work in Nuffield Department of Medicine Research Building and Chinese Academy of Medical Sciences Oxford Institute and will have the opportunity to interface with a network of collaborators in Oxford, UK and internationally to translate their data to the wider biomedical community.

Key Publications

1) Harris et al 2024. Oxygen-dependent histone lysine demethylase 4 restricts hepatitis B virus replication. PMID: 38325742. DOI: 10.1016/j.jbc.2024.105724
2) Cross et al 2024.Characterisation of HBV and co-infection with HDV and HIV through spatial transcriptomics. PMID: 39149129. DOI: 10.1136/egastro-2024-100067
3) Mukherji et al 2024. An atlas of the human liver diurnal transcriptome and its perturbation by hepatitis C virus infection. PMID: 39209804. DOI: 10.1038/s41467-024-51698-8
4) Zang et al 2022. Variations in dynamic tumor-associated antigen-specific T cell responses correlate with HCC recurrence after thermal ablation. PMID: 36618423. DOI: 10.3389/fimmu.2022.982578
5) Zakeri et al 2022. Characterisation and induction of tissue-resident gamma delta T-cells to target hepatocellular carcinoma. PMID: 35296658. DOI: 10.1038/s41467-022-29012-1

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3.12 A new role for hypoxia-signalling in post-transcriptional editing of viral RNAs and host inflammatory responses.

Prof. Jane McKeating, Dr. Peter Wing, Dr. Chunxiao Song & Prof. Jan Rehwinkel

Contact Email Address:

jane.mckeating@ndm.ox.ac.uk;

peter.wing@ndm.ox.ac.uk;

chunxiao.song@ludwig.ox.ac.uk;

jan.rehwinkel@ndm.ox.ac.uk

Project Overview

Respiratory viral infections in humans are responsible for a significant proportion of global deaths, approximately 4.25 million/year, mostly in children and older adults. Respiratory syncytial virus (RSV) is the leading cause of infant hospitalisation worldwide, infecting approximately 34 million children each year and a major cause of morbidity and mortality in elderly and immunosuppressed adults. The COVID-19 pandemic has highlighted the importance of understanding fundamental host processes that viruses exploit to infect the respiratory tract. One important factor to consider is local oxygen levels that can activate hypoxia inducible factors (HIFs) that we have reported restricts RSV and SARS-CoV-2 RNA replication via poorly understood mechanisms. Collectively, these studies highlight the gaps in our knowledge on cell-type dependent HIF-signalling responses and their role in orchestrating tissue-specific responses to viral infection and environmental stresses.

RNA modifications play a key role in regulating innate immune responses against a wide range of viruses. N^6-methyladenosine (m^6A) modification of RSV and SARS-CoV-2 RNA impacts transcript half-life, translation and evasion of innate signalling. Two key observations from our laboratory show a role for hypoxia to:

1) enhance host innate sensing of RSV and to activate interferon-stimulated gene expression;
2) inhibit m^6A-RNA levels via activating the demethylase pathway. **We hypothesise a central role for HIFs as rheostats that regulate the post-transcriptional machinery required for RNA viruses to replicate and evade innate sensing.**

Importantly, other types of RNA modifications exist including: 5-methylcytidine, adenosine-inosine editing (A-to-I editing), N^1-methyladenosine and pseudouridine, however, their function has remained elusive due to the lack of accurate detection methods. Our recent development of a quantitative and sensitive method to identify many of these modifications provides exciting opportunities to assess their role in the viral life cycle. At the present time there are limited antiviral therapies for treating these clinically important infections and given the well-recognised ability of these viruses to evolve resistance to immune and anti-viral therapies, there is an urgent need to develop new therapeutic approaches. This emerging field of RNA epigenetics offers fundamental insights into the viral life cycle and exciting new therapeutic opportunities.

Key words: RNA, epitranscriptome, immunology, virology.

Disease Relevance

Respiratory viral infections in humans are responsible for a significant proportion of global deaths, approximately 4.25 million/year, mostly in children and older adults. Respiratory syncytial virus (RSV) is the leading cause of infant hospitalisation worldwide, infecting approximately 34 million children each year and a major cause of morbidity and mortality in elderly and immunosuppressed adults.

Key Technology

- High resolution multiplex imaging of viral and host RNA in experimental model systems and infected tissues along with cell-cell network analysis (MuSpAn)
- RNA epigenetics - direct RNA sequencing methods to quantify post-transcriptional modifications along with bioinformatic analysis.

Training Opportunities

The student will join a dynamic and lively team of biologists in the McKeating, Wing, Song and Rehwinkel laboratories that bring complementary expertise in hypoxic biology, high resolution imaging of viral RNAs, RNA epigenetics and innate signalling. Importantly this project is supported by a collaboration with clinical respiratory immunologist Daniela Ferreira. This interdisciplinary project will provide a unique training environment to gain expertise in viral RNA biology and host innate responses to respiratory viral infections. Transferable skills include oral presentations at joint lab meetings, critical review of published scientific literature by contributing to journal clubs and scientific writing by reviewing and drafting manuscripts for publication. The student will work in Nuffield Department of Medicine Research Building and Chinese Academy of Medical Sciences Oxford Institute and will have the opportunity to interface with a network of collaborators in Oxford, UK and internationally to translate their data to the wider biomedical community.

Key Publications

1) Xu *et al* 2024. Absolute quantitative and base-resolution sequencing reveals comprehensive landscape of pseudouridine across the human transcriptome. PMID: 39349603. DOI: 10.1038/s41592-024-02439-8
2) Zhuang *et al* 2023. Hypoxia inducible factors inhibit respiratory syncytial virus infection by modulation of nucleolin expression. PMID: 38261926 DOI: 10.1016/j.isci.2023.108763
3) Tsukuda et al 2024. The N6-methyladenosine demethylase ALKBH5 regulates the hypoxic HBV transcriptome. PMID: 38227578. DOI: 10.1371/journal.ppat.1011917
4) Wing *et al* 2022. Hypoxia inducible factors regulate infectious SARS-CoV-2, epithelial damage and respiratory symptoms in a hamster COVID-19 model. PMID: 36067210 DOI: 10.1371/journal.ppat.1010807
5) Arnaiz *et al* 2021. Hypoxia regulates endogenous double-stranded RNA production *via* reduced mitochondrial DNA transcription. PMID: 34900733. DOI: 10.3389/fonc.2021.779739

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4. Vaccine and Antibodies

4.1 Pandemic influenza – can we protect older adults?

Prof. Teresa Lambe & Prof. Katrina Pollock

Contact Email Address:

teresa.lambe@paediatrics.ox.ac.uk;

katrina.pollock@paediatrics.ox.ac.uk

Project Overview

The current H5N1 avian influenza outbreak (strain 2.3.4.4b) is of growing international concern. The detection of virus in individuals with no known contact with dairy or avian livestock (where virus was first detected) suggests the worrying possibility of human-to-human transmission. Historically, the case fatality rate (CFR) for H5 and other emergent influenza strains can be as high as 60% in humans. Thankfully, cases of the current H5N1 strain have been largely mild, but hospitalisations have occurred. However, it is older adults, those with co-morbidities and younger children who are most at risk to succumbing to influenza infection — populations that have not yet been exposed during the current outbreak. Therefore, there is major concern that disease severity will increase if the virus begins to circulate more widely, or becomes better adapted for human-to-human transmission. Data on current population immunity and potency of current vaccines are desperately needed to understand the scope of the threat.

Pre-existing immunity can afford some protection against novel influenza strains, as was observed in older adults during the 2009 H1N1 pandemic. This was largely thought to be due to exposure to H1 strains that were circulating pre-1957. However, it is unknown if pre-existing immune responses, such as circulating antibody and memory B cells, exist in vulnerable cohorts that can readily target the currently circulating H5N1 2.3.4.4b strain. Furthermore, regulation of the immune processes in the lymph node by vaccination that can potently trigger broad protection against novel influenza strains are incompletely understood. These are the knowledge gaps we will address in our proposed project, directly informing if pre-existing immunity against H5 and emergent influenza strains exist in vulnerable cohorts and how such immunity can be induced to a level which could protect in the face of a H5N1 pandemic.

We are uniquely placed to address these outstanding questions with ongoing clinical trials spanning some thirty years and serological samples stored which would allow us to map the evolving landscape of immunity against H5 and other emergent influenza strains across age (paediatric, adult and older adult). In addition, we have bespoke sample-sets which facilitate an in-depth analysis of the immunological drivers underpinning heterosubtypic and cross-clade immunity against emergent influenza strains following seasonal influenza vaccination e.g. sample sets from 2023-2024 and 2024-2025 in older and younger adults with unique paired biological samples e.g. cells from lymph nodes (the site of immune activation), blood samples (circulating immune cells), with plasma and sera (samples to detect antibody responses).

We are experienced in combining systematic and routine assays (e.g. ELISA, systems serology (isotype, subclass and Fc function)) with bespoke innovative approaches (scRNAseq, TCR and BCR mapping) to delineate and define immunity against a wide range of influenza strains. In this manner, we will be able to detect and describe immunity against emergent influenza strains in paediatric, adult and older adults. Whilst also identifying the biological

pathways to target through vaccination to protect vulnerable populations against pandemic influenza. Importantly the outputs will directly inform vaccination strategies, vaccine design and immunisation priorities against the spread of H5N1 and other emergent influenza strains.

Disease Relevance

H5N1 or avian influenza continues to be of international concern given the global transmission in wild birds and detection of H5 in U.S. dairy cows and more recently the detection of virus in individuals with no known contact with diary or avian livestock. Generally the current H5N1 2.3.4.4b strain results in mild disease, however some cases have resulted in hospitalisation and virulence is likely to increase as the virus circulates more widely in humans. Importantly the case fatality rate (CFR) for H5 and other emergent influenza strains can be as high as 60% in humans. Approximately 20% of the protein consumed in developing countries come from poultry. A report by the FAO totalled economic losses caused by avian influenza in South East Asia up to 2005 around US$10 billion. This had the greatest impact on small scale commercial and backyard producers. Such is the epidemic and pandemic threat of the current circulating H5 strain that certain countries (e.g. Finland) have taken to prophylactically vaccinating workers in farms where avian influenza has previously been detected and spread easily (poultry and fur farms)

Key Technology

Specific training will be available in

- Vaccine design
- Preclinical models
- Immunology
- Molecular Biology
- Clinical trials

Facilitated through the following methodologies

- Systems serology (ELISA, neutralisation, Fc binding, Isotype, Subclass detection)
- ELISpot, Flow cytometry, prliferation assays
- ’omic analysis including scRNAseq, bulkseq, BCR and TCR clonotype analysis
- Vaccine design, development and testing.

Training Opportunities

As a doctoral student, your primary focus will be your research, with the opportunity to develop an original research project under the guidance of your supervisor(s). The department and University will also provide training to all DPhil students that focuses on developing research and professional skills.

You will be part of a vibrant research community that includes an active set of events, including seminars and workshops. You will have the opportunity to present and discuss your work informally in day-to-day meetings and by attending seminars/workshops in the department and at conferences. Involvement in public engagement outreach activities is strongly encouraged and specific training will be provided. The supervisors' groups operate under a strong ethos of equality, diversity and inclusion.

Our activities extend from basic and advanced immunological research into first-in-human clinical trials and studies of vaccine efficacy. This project will provide a broad range of transferable skills, with a unique insight into translational research. It will also provide opportunities for interaction with researchers, clinicians and staff across a broad range of scientific disciplines related to immunology and vaccinology:

Specific training will be available in：

- Vaccine design
- Preclinical models
- Immunology
- Molecular Biology
- Clinical trials

Key Publications

1) Saunders JE, Gilbride C, Dowall S, Morris S, Ulaszewska M, Spencer AJ, Rayner E, Graham VA, Kennedy E, Thomas K, Hewson R, Gilbert SC, Belij-Rammerstorfer S, Lambe T. Adenoviral vectored vaccination protects against Crimean-Congo Haemorrhagic Fever disease in a lethal challenge mode. *EBioMedicine.* 2023 Apr;90:104523. doi: 10.1016/j.ebiom.2023.104523.
2) Sharpe HR, Provine NM, Bowyer GS, Moreira Folegatti P, Belij-Rammerstorfer S, Flaxman A, Makinson R, Hill AV, Ewer KJ, Pollard AJ, Klenerman P, Gilbert S, Lambe T. CMV-associated T cell and NK cell terminal differentiation does not affect immunogenicity of ChAdOx1 vaccination. *JCI Insight*. 2022 Mar 22;7(6):e154187. doi: 10.1172/jci.insight.154187.
3) Costa Clemens SA, Weckx L, Clemens R, Almeida Mendes AV, Ramos Souza A, Silveira MBV, da Guarda SNF, de Nobrega MM, de Moraes Pinto MI, Gonzalez IGS, Salvador N, Franco MM, de Avila Mendonça RN, Queiroz Oliveira IS, de Freitas Souza BS, Fraga M, Aley P, Bibi S, Cantrell L, Dejnirattisai W, Liu X, Mongkolsapaya J, Supasa P, Screaton GR, Lambe T, Voysey M, Pollard AJ; RHH-001 study team. Heterologous versus homologous COVID-19 booster vaccination in previous recipients of two doses of CoronaVac COVID-19 vaccine in Brazil (RHH-001): a phase 4, non-inferiority, single blind, randomised study. *Lancet.* 2022 Feb 5;399(10324):521-529. doi: 10.1016/S0140-6736(22)00094-0.
4) Feng S, Phillips DJ, White T, Sayal H, Aley PK, Bibi S, Dold C, Fuskova M, Gilbert SC, Hirsch I, Humphries HE, Jepson B, Kelly EJ, Plested E, Shoemaker K, Thomas KM, Vekemans J, Villafana TL, Lambe T, Pollard AJ, Voysey M; Oxford COVID Vaccine Trial Group. Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. *Nat Med.* 2021 Sep 29. doi: 10.1038/s41591-021-01540-1.
5) Flaxman A, Marchevsky NG, Jenkin D, Aboagye J, Aley PK, Angus B, Belij-Rammerstorfer S, Bibi S, Bittaye M, Cappuccini F, Cicconi P, Clutterbuck EA, Davies S, Dejnirattisai W, Dold C, Ewer KJ, Folegatti PM, Fowler J, Hill AVS, Kerridge S, Minassian AM, Mongkolsapaya J, Mujadidi YF, Plested E, Ramasamy MN, Robinson H, Sanders H, Sheehan E, Smith H, Snape MD, Song R, Woods D, Screaton G, Gilbert SC, Voysey M, Pollard AJ, Lambe T; Oxford COVID Vaccine Trial group. Reactogenicity and immunogenicity after a late second dose or a third dose of ChAdOx1 nCoV-19 in the UK: a substudy of two randomised controlled trials (COV001 and COV002). *Lancet.* 2021 Sep 11;398(10304):981-990. doi: 10.1016/S0140-6736(21)01699-8.

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4.2 Estimating Vaccine Efficacy Using Serological Data

Prof. Xinxue Liu & Prof. Sir Andrew Pollard

Contact Email Address:

xinxue.liu@paediatrics.ox.ac.uk;

andrew.pollard@paediatrics.ox.ac.uk

Project Overview

In 2017, the Bill & Melinda Gates Foundation funded the Typhoid Vaccine Acceleration Consortium (TyVAC), a collaborative project aimed at advancing the introduction of new typhoid conjugate vaccines (TCVs). TyVAC is a partnership between the Center for Vaccine Development and Global Health (CVD) at the University of Maryland School of Medicine, the Oxford Vaccine Group at the University of Oxford, and PATH, an international nonprofit organization. Its goal is to reduce the burden of typhoid in countries eligible for support from Gavi, the Vaccine Alliance, through an integrated approach.

TyVAC supported three large randomised controlled trials (RCTs) in Africa and Asia, vaccinating between 20,000 and 60,000 children, to assess the efficacy/effectiveness of a single dose of TCV in endemic regions. All three trials demonstrated similar protection levels, ranging from 79% to 85%. These results, published between 2019 and 2021 in The New England Journal of Medicine and The Lancet, provided robust evidence supporting the global introduction of TCVs. Two of three trials (Nepal and Bangaldesh) are ongoing, focusing on long-term protection to inform decisions about vaccine schedules. The 3-5 years protection result in Bangaldesh has been accepted by the Lancet and the Nepal data will be analysed in 2025.

One of the challenges in vaccine development, as seen in TyVAC, is demonstrating efficacy in large RCTs. This difficulty results from the low incidence of typhoid, which requires large sample sizes (typically over 20,000 participants) to confirm vaccine efficacy. The gold standard for typhoid diagnosis is the culture of S. Typhi from the blood of symptomatic patients. Because blood culture-confirmed typhoid cases are rare, alternative methods are needed to accelerate vaccine development. Previous studies have shown that vaccine efficacy can be estimated using immunogenicity data, by modeling serologically defined infections and comparing incidence rates between randomised groups in a clinical trial.

In each of the three TyVAC trials, an immunogenicity cohort of approximately 1,500 participants was followed at regular intervals—day 0, 28 days, 18 months, 24 months, 48 months, and 60 months post-vaccination—to monitor antibody kinetics through blood samples. This project aims to use the serological data generated by TyVAC to:

- Model the serological incidence based on paired immunogenicity data from immunogencity cohorts (~4500 in total) in three countries;
- Estimate the vaccine protection by serologically defined infections and compare with vaccine protection estimated using blood-culture-confirmed infections.

Disease Relevance

Typhoid fever continues to place a significant disease burden on low- and middle-income countries marked by inadequate sanitation and limited access to clean water. There are estimated to be 9 million cases and 110,000 deaths annually worldwide. The escalation of

antimicrobial resistance, notably underscored by the emergence of extensively drug-resistant strains in Asia, has reduced available treatment options and increased the public health threat from this disease. The World Health Organisation (WHO) recommended vaccines as an important tool in typhoid prevention and control.

The current recommendation by the WHO is a single dose of TCV for infants and children from 6 months of age. With support from GAVI, the Vaccine Alliance, an international organisation to improve access to new and underused vaccines for children living in the world's lowest-income countries, Pakistan, Nepal, Liberia, and Zimbabwe became the first countries to introduce TCV nationwide in Asia and Africa between 2019-2022 to reduce the disease burden.

Key Technology

Since this project requires strong numeric skills to conduct the statistical model and analysis. The successful candidate for this project will be expected to satisfy the following criteria:

- Demonstrated experience and ability in statistics or epidemiology
- Experience in the use of statistical software
- Excellent communication skills
- Demonstrated research interests in infectious disease and clincial trial methodology

Training Opportunities

The successful candidate will be based at the statistics and epidemiology group (a team of 13 statisticians, bioinformaticians, and epidemiologists) at OVG, department of paediatrics. Besides all the free training at OVG, the department, and the wider Oxford University, the department and OVG have separate funding schemes to support students in developing and training.

The successful candidate will also have training opportunities in the design, conduct, and analysis of RCTs and observational studies.

Key Publications

1) Qadri F, et al. Five-Year Vaccine Protection Following a Single Dose of Vi-Tetanus Toxoid Conjugate Vaccine in Bangladeshi Children: A Cluster Randomised Trial. The Lancet. 2024, accepted.
2) Qadri, Firdausi et al. “Protection by vaccination of children against typhoid fever with a Vi-tetanus toxoid conjugate vaccine in urban Bangladesh: a cluster-randomised trial.” Lancet (London, England) vol. 398,10301 (2021): 675-684. doi:10.1016/S0140-6736(21)01124-7
3) Shakya, Mila et al. “Phase 3 Efficacy Analysis of a Typhoid Conjugate Vaccine Trial in Nepal.” The New England journal of medicine vol. 381,23 (2019): 2209-2218. doi:10.1056/NEJMoa1905047
4) Patel, Priyanka D et al. “Safety and Efficacy of a Typhoid Conjugate Vaccine in Malawian Children.” The New England journal of medicine vol. 385,12 (2021): 1104-1115. doi:10.1056/NEJMoa2035916
5) Voysey M, Pollard AJ. Seroefficacy of Vi Polysaccharide-Tetanus Toxoid Typhoid Conjugate Vaccine (Typbar TCV). Clin Infect Dis. 2018 Jun 18;67(1):18-24. doi: 10.1093/cid/cix1145. PMID: 29351594.

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4.3 Enhancing delivery of nucleic acid vaccines

Prof. Dame Sarah Gilbert & Prof. Bruno Douradinha

Contact Email Address:

sarah.gilbert@ndm.ox.ac.uk;

bruno.douradinha@ndm.ox.ac.uk

Project Overview

Nucleic acid-based vaccines, including DNA and mRNA platforms, represent highly effective approaches to immunisation. DNA vaccines deliver plasmid DNA encoding specific antigens, which must enter the nucleus for transcription to occur before antigen expression. In contrast, mRNA vaccines introduce messenger RNA directly into the cytoplasm, where it is translated into protein antigens without the need for nuclear entry. Both platforms have shown tremendous promise, particularly during the COVID-19 pandemic, where both DNA and mRNA vaccines demonstrated their efficacy in preventing severe disease. Their safety profiles, ease of production, and ability to induce potent immune responses make them highly valuable. However, challenges persist, particularly in optimising delivery systems and enhancing antigen expression. Lipid nanoparticles (LNPs) are commonly used to improve the uptake and stability of both DNA and mRNA vaccines, although further optimisation is necessary to achieve more efficient delivery and stronger immune activation.

Molecular adjuvants also offer an additional strategy to enhance the efficacy of nucleic acid vaccines. Immunostimulatory molecules can be co-delivered with nucleic acids or formulated within LNPs to boost antigen expression and enhance immune system activation. Integrating molecular adjuvants into vaccine formulations may significantly improve both the potency and breadth of immune responses.

The proposed project aims to explore and optimise nucleic acid delivery strategies. These strategies will be applied to both DNA and mRNA vaccines, where relevant, to evaluate their potential to enhance the overall efficacy of nucleic acid-based vaccines. By addressing key challenges in antigen delivery and expression, this research seeks to develop more efficient and scalable platforms for vaccines and gene therapies.

The primary goal is to improve the design and delivery of nucleic acid vaccines, extending their application across a range of clinical contexts. This project will focus on refining both existing and novel technologies, taking a practical and well-grounded approach to advancing the next generation of nucleic acid vaccine platforms.

Disease Relevance

Nucleic acid vaccines are powerful tools in combating a wide range of diseases, making them especially useful against infectious diseases in a pandemic setting. mRNA vaccines like Pfizer-BioNTech (Comirnaty) and Moderna (Spikevax), along with the DNA vaccine ZyCoV-D, were crucial in the fight against COVID-19. These platforms have also been applied to diseases such as Zika, Dengue, and HIV, demonstrating their versatility against these diseases at preclinical level and in clinical trials. Both DNA and mRNA vaccines are highly effective at generating strong humoral and cellular immune responses, making them ideal for pathogens requiring sustained immunity.

Beyond infectious diseases, these immunisation platforms are being explored for cancer immunotherapy, offering potential for targeting tumour cells and genetic disorders. However,

optimising delivery systems is essential to enhance antigen expression and immune activation, critical for treating chronic infections and cancers.

This project focuses on improving delivery strategies for DNA and mRNA vaccines, aiming to develop more effective and scalable solutions for both infectious and non-communicable diseases like cancer, advancing global health efforts.

Key Technology

The project employs a range of key technologies to enhance the delivery and efficacy of DNA and mRNA vaccines. Both platforms hold significant potential due to their versatility, rapid production capabilities, and ability to elicit robust immune responses. However, optimising delivery systems and improving antigen expression remain critical challenges.

Several technologies will be explored, either individually or in combination. LNPs, for example, are crucial for protecting nucleic acids and enhancing cellular uptake. LNPs facilitate the efficient delivery of mRNA and DNA into cells, where the genetic material can be translated into the target antigen. Optimising the composition of the single components of LNPs is key to improving the delivery efficiency of nucleic acid vaccines. Additionally, polymeric nanoparticles, which are biodegradable polymers, encapsulate nucleic acids to enhance stability and regulate the controlled release of the vaccine. These polymeric nanoparticles offer the advantage of targeted delivery and prolonged antigen expression. Cationic lipids and polymers represent another approach, as these molecules form complexes with nucleic acids to create positively charged particles that interact with the negatively charged cell membrane, thereby enhancing cellular uptake.

Additionally, molecular adjuvants offer a complementary approach to further enhance the delivery and immune activation of nucleic acid vaccines. These adjuvants can be co-delivered with DNA or mRNA to boost immune recognition and antigen expression. Incorporating molecular adjuvants into the nanoparticle formulations allows for enhanced activation of immune pathways, thereby improving vaccine uptake and the subsequent adaptive immune response. The inclusion of adjuvants may also help to fine-tune the immune response, leading to longer-lasting protection and higher efficacy across diverse populations.

These innovations aim to address the limitations of current nucleic acid vaccines, improving both delivery and antigen expression to ensure a more robust immune response. By integrating a range of cutting-edge technologies, the project seeks to develop scalable and effective vaccine platforms applicable to a broad spectrum of diseases, including infectious diseases and cancer.

Training Opportunities

As part of our research team, the PhD student will join a dynamic, collaborative environment that fosters both academic and professional development. Our team offers extensive expertise in vaccinology, providing a rich platform for learning and innovation. Regular meetings with supervisors and fellow researchers ensure continuous feedback and support, allowing students to enhance their technical skills and grow professionally. The team emphasises hands-on experience, encouraging members to actively contribute to ongoing projects while developing their own research ideas.

The program also offers numerous opportunities for students to engage in academic activities beyond their research, including seminars, workshops, and informal discussions where they can share progress and receive constructive input from faculty and peers. Public outreach is a key component of the experience, with students encouraged to participate in engagement

activities aimed at improving science communication skills. Specialised training in these areas will be provided to ensure effective involvement.

Through this program, students will gain exposure to various aspects of vaccinology, including molecular biology, vaccine design, and clinical research. Under the mentorship of their supervisors, they will develop an original research project that aligns with their interests and expertise. The program also focuses on developing transferable skills, equipping students with the tools needed to succeed in future careers, whether in academia, industry, or public health. The department provides targeted training to ensure students build a solid foundation in both research methodologies and professional development.

The team's commitment to equality, diversity, and inclusion ensures a supportive environment where all students are encouraged to thrive, contributing to both the scientific community and broader societal goals.

Key Publications

1) Guimaraes, L.C., Costa, P.A.C., Scalzo Júnior, S.R.A. et al. Nanoparticle-based DNA vaccine protects against SARS-CoV-2 variants in female preclinical models. Nat Commun, 15, 590 (2024).
2) Cagigi, A., and Douradinha, B. Have mRNA vaccines sentenced DNA vaccines to death? Expert Rev Vaccines, 22, 1154-1167 (2023).
3) Voysey, M., Clemens, S.A.C., Madhi, S.A. et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. Lancet, 397, 99–111 (2021).
4) Tenchov, R., Bird, R., Curtze, A.E., & Zhou, Q. Lipid nanoparticles─From liposomes to mRNA vaccine delivery, a landscape of research diversity and advancement. ACS Nano, 15, 16982–17015 (2021).
5) Puksuriwong, S., Ahmed, M.S., Sharma, R., Krishnan, M., Leong, S., Lambe, T., McNamara, P.S., Gilbert, S.C., & Zhang, Q. Modified Vaccinia Ankara-vectored vaccine expressing nucleoprotein and matrix protein 1 (M1) activates mucosal M1-specific T-cell immunity and tissue-resident memory T cells in human nasopharynx-associated lymphoid tissue. J Infect Dis, 222, 807-819 (2020).

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4.4 Effects of adaptive immunoregulation on vaccine responses using human splenic organoids

Prof. Fadi Issa, Dr. Sushma Shankar & Dr. Nicholas Provine

Contact Email Address:

fadi.issa@nds.ox.ac.uk;

sushma.shankar@nds.ox.ac.uk;

nicholas.provine@ndm.ox.ac.uk

Project Overview

Annual influenza vaccination is a critical public health measure, yet vaccine efficacy varies among individuals. Recent studies suggest that Tregs and Bregs play significant roles in attenuating protective immune responses:

***Tregs**:*

- In mouse models, viral antigen-specific Tregs have been shown to reduce the magnitude of protective anti-viral immunity.
- Human studies indicate that repeated influenza vaccination correlates with higher frequencies of vaccine-specific Tregs and lower vaccine-induced antibody responses.
- A lower pre-existing Treg frequency is associated with higher post-vaccination antibody titers.

Bregs:

- Bregs can suppress viral antigen-specific $CD8^+$ T cell responses and inhibit antigen presentation and T follicular helper cell differentiation.
- Infections like HBV and HIV-1 show that Bregs induce antigen-specific Tregs.
- Mouse Bregs can regulate B cell differentiation into plasma cells through the silencing of PRDM1.
- Elevated levels of circulating Bregs are associated with HBV vaccine non-responders.

However, the specific effects of Tregs and Bregs on T-cell receptor (TCR) and B-cell receptor (BCR) diversity, antibody affinity, and protective antibody titers in the context of viral vaccination remain unclear.

Human splenic organoids:

Traditional murine models have limitations in replicating essential features of human adaptive immunity, such as affinity maturation, class switching, and responses to adjuvants. Human splenic organoids present an innovative platform to study germinal center reactions in a human-relevant context. These organoids support antigen-specific somatic hypermutation, affinity maturation, and class switching of B cells in response to infection and vaccination.

We propose to evaluate the effects of human Tregs and Bregs on influenza vaccine responses within the germinal centre, using novel, human splenic organoids derived from spleens donated by deceased human organ donors (IRAS ID: 339337).

In Aim 1, we will determine the influence of Tregs and Bregs on antibody responses to influenza vaccination within the splenic organoids. We will generate human splenic organoids, ensuring they mimic native splenic architecture and support germinal center formation. We will introduce seasonal influenza vaccine antigens and measure neutralising antibody titers. To assess the specific roles of Tregs and Bregs, we will isolate these cells from the organoids and perform depletion and supplementation experiments.

For Aim 2, we will assess the impact of repeated vaccination on Treg and Breg repertoires within the splenic organoids. By subjecting the organoids to multiple rounds of influenza antigen exposure, we will simulate the effects of repeated annual vaccinations. We will monitor changes in the frequency and function of Tregs and Bregs over time. High-throughput sequencing of TCRs and BCRs will assess repertoire diversity, clonal expansion, and changes in antigen specificity. We aim to identify patterns in how repeated exposure affects regulatory cell repertoires and functions.

In Aim 3, we will investigate the spatial organisation of immune cells within germinal centers and how Tregs and Bregs modulate this architecture. Using immunofluorescence staining and confocal microscopy, we will visualise the three-dimensional arrangement of T and B cells within the organoids. We will assess how the presence or absence of these regulatory cells affects the structural organisation of germinal centers.

Disease Relevance

Vaccination is a vital strategy to protect populations from infectious disease. However, as highlighted in the recent pandemic and by ongoing public health schemes, a significant proportion of the population mount poor responses to vaccination. Indeed, recent evidence suggests repeated influenza vaccination results in poorer sequential protective humoral responses in association with increasing immunoregulation.

There is a growing interest in modulating endogenous immunoregulatory populations to improve the immunogenicity and effectiveness of vaccines. However, immunoregulatory mechanisms and effects on TCR/BCR repertoire diversity, antibody affinity and the immunomodulatory dynamics of repeated vaccination remain unclear. The use of novel in vitro platforms to model complex human adaptive responses promises to transform the way in which the human immune system is interrogated and understood. Human splenic organoids derived from diverse donor cohorts can enable the evaluation of the impact of demographic parameters including age, ethnicity and sex upon vaccine responses, providing vital pre-clinical guidance prior to clinical trials.

Whilst this project focuses on influenza vaccine responses, an improved understanding of the principles of immunoregulation upon vaccination will provide proof of concept and may have wider impact in the field of vaccination in a multitude of viral infection contexts, including HIV-1, HBV and SARS-CoV-2.

Key Technology

Human Splenic Organoids: Single-cell suspensions from spleens (n=50 donors) will be plated into transwells alongside antigens of interest, including multiple strains of live attenuated influenza vaccine (LAIV) or subunit influenza vaccine (IIV).

Fluorescence-activated cell sorting: Depletion experiments will assess roles of immunoregulatory cells on antigen-specific cellular and humoral responses within splenic organoids, in response to LAIV/IIV stimulation. Concomitant removal of high-affinity

hemagglutinin $(HA)^+$ B cells and non-naïve B cells from the splenic cell pool prior to LAIV stimulation, will enable assessment of new high-affinity HA^+ B cells. This will provide direct evidence of the effects of immunoregulatory cells on affinity maturation.

scRNASeq, TCR & BCR sequencing: Single cell sequencing of BCRs from high affinity HA^+ B cells post-LAIV stimulation will evaluate affinity maturation and isotype switching. IgH sequencing will analyse BCR repertoires in naive vs. affinity matured B cells, including Bregs, in response to LAIV/ILV stimulation +/- immunoregulatory cell depletion. Longitudinal TCR sequencing will clarify concomitant antigen-specific effector/Treg repertoires.

Single cell spatial transcriptomics: To delineate changes in GC organisation and cellular interactions at a single cell level, in response to vaccination +/- immunoregulation.

CRISPR: Gene knockouts of critical Treg and Breg transcription factors and pathways within splenic cell pools prior to LAIV/ILV stimulation will define mechanisms involved in the immunoregulation of vaccine responses.

Flow Cytometry: To assess baseline and emerging phenotypes of follicular helper T cells (Tfh), effector, naïve and memory T and B cells, Treg, Breg, plasma cells, pre-Germinal Centre (GC) and GC B cells pre- and post-stimulation with LAIV/ILV. Activation-induced cytidine deaminase levels will help determine somatic hypermutation and class switching. Activation-induced marker assays will measure antigen-specific T cell responses in an HLA-agnostic manner.

Affinity binding, neutralising, ELISA, ELISpot assays: To identify antigen-specific antibody secreting cells and immunoglobulin, and changes in affinity binding in relation to immunoregulatory cells.

Training Opportunities

Cell culture, ELISA, ELISpot & Affinity binding assays: The Translational Research & Immunology Group (TRIG), where this project will be based, has extensive experience in primary human cell culture and antibody-based assays. There is ample access to large tissue culture facilities. A team of PIs and post-doctoral researchers will provide hands-on training to support the student.

Gene editing: Multiple gene editing technologies and their application in primary human lymphocytes, including guide RNA design, CRISPR/Cas9 and lentiviral transduction, are well-established within TRIG. Dedicated post-doctoral researchers and PIs will provide training and supervision.

Single cell spatial transcriptomics: TRIG has extensive experience in single cell sequencing and spatial transcriptomics. TRIG has ready access to 10x Genomics platforms, including Xenium and Visium HD, within the department. Dedicated post-doctoral researchers will provide training and supervision.

Bioinformatics: Alongside post-doctoral-led training in the analysis of large transcriptomic datasets within TRIG, the DPhil candidate will be encouraged to apply to the Oxford Biomedical Data Science Training Programme. This 6-week course provides foundation training in R for data science, genomics, single cell RNA sequencing analysis and the Linux command line.

Flow Cytometry & FACS sorting: TRIG has multiple flow cytometers and cell sorters to support this project. The DPhil candidate will receive detailed training by post-doctoral researchers and project supervisors regarding flow cytometry principles as well as practical supervision. TRIG has close links with the Experimental Medicine Division Flow Cytometry Facility, which regularly provides tutorials, including to TRIG-based researchers.

Presentation skills & critical appraisal: TRIG hosts weekly laboratory meetings and journal clubs, where researchers present and critically appraise their work as well as other advances in the literature.

Weekly supervisions: The DPhil candidate will meet with project supervisors on a minimum weekly basis, to discuss data, progress and challenges. These meetings are designed to ensure professional and pastoral support for the student.

Key Publications

1) Wagar LE et al. Modeling human adaptive immune responses with tonsil organoids. Nat Med. 2021 Jan;27(1):125-135. doi: 10.1038/s41591-020-01145-0. Epub 2021 Jan 11. PMID: 33432170; PMCID: PMC7891554.
2) Shankar S et al. Ex vivo-expanded human $CD19^{+}TIM\text{-}1^{+}$ regulatory B cells suppress immune responses in vivo and are dependent upon the TIM-1/STAT3 axis. Nat Commun. 2022 Jun 3;13(1):3121. doi: 10.1038/s41467-022-30613-z. PMID: 35660734; PMCID: PMC9166804
3) Lin PH et al. Association of vaccine-specific regulatory T cells with reduced antibody response to repeated influenza vaccination. Eur J Immunol. 2023 Dec;53(12):e2350525. doi: 10.1002/eji.202350525. Epub 2023 Sep 29. PMID: 37713727
4) Mauri C et al. Human regulatory B cells in health and disease: therapeutic potential. J Clin Invest. 2017 Mar 1;127(3):772-779. doi: 10.1172/JCI85113. Epub 2017 Mar 1. PMID: 28248202; PMCID: PMC5330739.
5) Lin PH et al. Vaccine-induced antigen-specific regulatory T cells attenuate the antiviral immunity against acute influenza virus infection. Mucosal Immunol. 2018 Jul;11(4):1239-1253. doi: 10.1038/s41385-018-0004-9. Epub 2018 Feb 21. PMID: 29467445.

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4.5 Study of antibody responses against emerging pathogens toward vaccine design, therapeutics and protection.

Prof. Gavin Screaton & Prof. Juthathip Mongkolsapaya

Contact Email Address:

gavin.screaton@medsci.ox.ac.uk;

juthathip.mongkolsapaya@well.ox.ac.uk

Project Overview

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

Our lab has studied a number of emerging viruses, including dengue, Zika, SARS-CoV-2, and Ebola for more than 20 years. Our work has contributed to understanding pathogenic mechanisms, generating diagnostic and therapeutic reagents, designing vaccines, and contributing to policy development. We have published several scientific articles in high-impact journals such as Nature, Science, Cell, Nature Immunology, Nature Communications, and Immunity. For example, during the COVID pandemic, we generated reagents that were involved in establishing a protocol to measure the antibody response, which has been used to monitor the immune status in the UK population. We have generated hundreds of monoclonal antibodies from infected and vaccinated individuals. We characterized their neutralization activities, cross-reaction among the variants, and biophysical properties. In combination with crystal and cryo-EM structures, we described the antigenic distance among the variants and how new emerging variants escaped from existing ones.

The general aim of the proposed D.Phil project is to apply our expertise to study new emerging pathogens, such as Dengue virus, Sudan Ebolavirus, which caused a large outbreak in Uganda at the end of 2022, Nipah virus, enterovirus, and Monkeypox virus,which WHO declares the outbreak a public health emergency of international concern.

Disease Relevance

When a new pathogen emerges and causes severe or life-threatening disease, it has the potential to initiate a major outbreak or even evolve into a global pandemic. The impact of such an event can be catastrophic, as it not only threatens public health but also overwhelms healthcare systems, disrupts economies, and places immense pressure on governments and international organizations. This threat becomes particularly alarming when there are no vaccines or treatments readily available, leaving the population vulnerable to the spread of the disease.

This project will serve as an exercise in preparing for future emerging diseases and potential pandemic outbreaks; whether it's a new strain of influenza, a novel coronavirus, or an entirely unknown virus.

Key Technology

The student will have an opportunity to receive hand-on training in a wide range of cutting-edge technologies, including:

- **Flow Cytometry and Single-Cell Sorting**: Mastering the use of flow cytometers for the precise sorting and analysis of individual cells, a critical technique for immunology, medical research, and cell biology.
- **Molecular Biology Techniques**: Gaining expertise in fundamental molecular biology methods such as DNA/RNA extraction, PCR amplification, and gel electrophoresis, which are essential for genetic analysis and manipulation.
- **Gene Editing**: Learning gene-editing technologies to perform targeted modifications in genomes, allowing for functional studies of genes and their roles in disease.
- **Cell Culture and Maintenance**: Acquiring skills in culturing and maintaining various cell lines, which is crucial for conducting in vitro experiments, and biological research.
- **Recombinant Protein Expression**: Training in the expression and purification of recombinant proteins using different systems such as bacterial, yeast, and mammalian cells, which is important for structural and functional protein studies.
- **Viral isolation and viral culture** are essential techniques in virology and medical researches that involve extracting and growing viruses from clinical or environmental samples. Training in these methods is crucial expecially for new emerging pathogens.
- **Reverse Genetics and Virus Generation**: Learning reverse genetic techniques to engineer both wild-type and mutated viruses, providing insights into viral pathogenesis, replication, and vaccine development.
- **Structural Biology**: Exposure to methods used in structural biology for determining the three-dimensional structure of proteins and viruses, which is key for understanding molecular interactions and drug design.

This comprehensive training will equip the student with a diverse set of skills that are highly applicable across multiple disciplines in the biomedical sciences.

Training Opportunities

The student will have the opportunity to join a highly experienced team with over 20 years of expertise in virology, immunology, and molecular biology. Under the mentorship of researchers and postdoctoral fellows, the student will receive comprehensive training in a wide range of cutting-edge techniques, including flow cytometry, gene editing, reverse genetics, viral isolation, and recombinant protein expression.

The student will gain experience in generating monoclonal antibodies from single human and mouse B cells. This process is critical for advancing research in immune response and developing targeted therapies for infectious diseases. The student will learn to isolate B cells, clone antibody genes, express and purify monoclonal antibodies, and analyse their properties, which are valuable tools for diagnostic, therapeutic applications and vaccine design.

Collaboration will be a key aspect of this experience. The student will work closely with our network of partners, including structural biologists based in Oxford, as well as collaborators across the UK and internationally. This interdisciplinary environment will expose the student to a broader scope of researches.

By the end of the training, the student will have acquired a well-rounded skill set, including not only laboratory techniques but also experience in collaborative research, project management, and scientific communication. This experience will prepare the student for a successful career in biomedical research, virology, immunology, or biotechnology, equipping him/her to contribute to critical areas such as vaccine development, therapeutic discovery, and pandemic preparedness.

Key Publications

1) Dejnirattisai, W. *et al.* The antigenic anatomy of SARS CoV-2 receptor binding domain. *Cell* 184, 2183-2200 e2122, doi:10.1016/j.cell.2021.02.032 (2021).
2) Dejnirattisai, W. *et al.* Reduced neutralisation of SARS-CoV-2 omicron B.1.1.529 variant by post-immunisation serum. *Lancet* 399, 234-236, doi:10.1016/S0140-6736(21)02844-0 (2022).
3) Rouvinski, A. *et al.* Recognition determinants of broadly neutralizing human antibodies against dengue viruses. *Nature* 520, 109-113, doi:10.1038/nature14130 (2015).
4) Fernandez, E. *et al.* Human antibodies to the dengue virus E-dimer epitope have therapeutic activity against Zika virus infection. *Nat Immunol* 18, 1261-1269, doi:10.1038/ni.3849 (2017).
5) Dejnirattisai, W. *et al.* Cross-reacting antibodies enhance dengue virus infection in humans. *Science* 328, 745-748, doi:10.1126/science.1185181 (2010).

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5. T cell Immunology

5.1 Decoding the Rules of T Cell Recognition of Antigens Using Integrative Deep Learning and Protein Language Models

Prof. Hashem Koohy & Prof. Tao Dong

Contact Email Address:

hashem.koohy@rdm.ox.ac.uk;

tao.dong@ndm.ox.ac.uk

Project Overview

Introduction

Cellular T cell immunity is a cornerstone of the adaptive immune system. The antigen-specific T cell response is initiated when the T cell receptor (TCR) interacts with a target antigen, presented as a short peptide by the major histocompatibility complex (MHC) or similar molecules on the surface of target cells. T cells play a critical role in combating diseases such as cancer and infections, but they are also implicated in the development of autoimmune disorders. Gaining a deeper understanding of what triggers these T cell responses could not only unravel the mechanisms behind immune activation but also advance T cell-based therapies.

Background

In recent years, we have witnessed the emergence of cutting-edge single-cell technologies capable of profiling multiple molecular readouts, including transcriptomics, TCR sequences, proteomics, and even spatial coordinates of T cells within tissues. These advances have generated an unprecedented amount of T cell data, much of which involves orphan TCRs (TCRs without known target antigens). This explosion of data has prompted researchers to apply advanced computational approaches like Protein Language Models (PLMs) and other AI strategies to map TCRs to their target antigens. While significant progress has been made in understanding the rules governing TCR-antigen interactions under various immunological conditions, a generalizable map that links TCRs to their cognate peptide-MHC (pMHC) complexes remains a formidable challenge in the field of digital immunology.

Project Objectives

This collaborative project aims to build upon our ongoing research that leverages single-cell data and harnesses the power of AI and deep neural networks (DNNs) to decode the fundamental principles of TCR interactions with target antigens. With a focus on CD4+ T cells (and possibly gamma-delta T cells), we aim to investigate the following key questions:

What are the shared features of immunogenic MHC class II peptides, and to what extent can these features explain T cell cross-reactivity?

What are the common characteristics of antigen-specific TCRs? Can these features be utilized to identify other TCRs with similar antigen specificity?

Methodology

A significant portion of this project will involve systematic literature review, data curation, and alignment to assemble high-quality datasets for analysis. This will provide the candidate with valuable data science skills tailored to the fields of immunology and medical research.

We will then develop and apply advanced PLMs, DNNs, and AI models, including deep generative models, attention-based learning, and self- or semi-supervised learning models. These models will generate low-dimensional interpretable latent spaces for downstream tasks, such as mathematical modeling, data interpretation, or synthetic data generation. This project offers a unique opportunity to explore cutting-edge AI methodologies in the context of immunology and medicine.

Applications and Impact

In the final stage of the project, we will apply the developed models to answer critical immunological questions, including:

1) *Autoimmunity:*
   - Identifying the self-antigens that regulatory CD4+ T cells react to.
2) *Cancer immunotherapy:*
   - Identifying both CD4+ and CD8+ patient-specific neoepitopes
   - De novo design or optimiztion of CAR-T and TCR-T cells
3) *Infectious diseases:*
   - Exploring the extent and source of T cell cross-protection and immunopathology in infections.

By leveraging integrative deep learning and protein language models, this project will contribute to solving some of the grand challenges in T cell biology, advancing both basic understanding and therapeutic applications.

Disease Relevance

Antigen-specific T cell activation is crucial for protecting the body from various pathological diseases and holds immense potential for understanding disease progression and therapeutic interventions. It is also a key driver of immunopathology, contributing to the onset of autoinflammatory and autoimmune disorders.

Our research, which focuses on developing ML/AI models to map TCRs to their target antigens, has significant relevance for several critical human diseases, including infectious diseases, cancer and cancer immunotherapy, as well as autoimmune and autoinflammatory conditions. The candidate can explore the following directions in disease relevance:

Infectious Diseases:

- Identify virus-specific T cells and explore how the virus-specific TCR repertoire correlates with disease outcomes and/or response to vaccinaitons.
- Identify viral epitopes targets by Tcells and investigate cross-reactivity features and protection mechanisms.
- Examine viral-host interactions at the tissue and cellular level.

Cancer and Cancer immunotherapy:

- Identify patient-specific cancer neoantigens
- Characterize circulating and tumour-infiltrating TCRs and their functional/phenotypic status.
- Guide de novo development and/or optimization of CAR-T cell strategies

Autoimmune/Autoinflammatory Diseases:

- Identify self-reactive tissue-resident TCRs and their functional/phenotypic status.
- Determine the self-peptides that trigger off target T cell response, and their underlying mechanisms.

Key Technology

The data utilized in our research are typically sourced from public repositories or generated through our collaborations with experimental partners.

These datasets are primarily derived from cutting-edge single cell and spatial transcriptomics sequencing, as well as advanced imaging technologies. They encompass a wide range of molecular readouts, including paired alpha-beta TCR sequencing, transcriptomic profiles, and proteomics. For some T cells with known antigen-specificity, information about their corresponding antigens is also available.

The candidate will have the opportunity to gain hands-on experience with these emerging state-of-the-art single cell technologies and the rich dataset they generate.

From a model development perspective, these large, high-dimensional datasets offer an excellent platform for leveraging groundbreaking AI/ML and Protein Language Models PLM. Prominent approaches include, but are not limited to:

- Deep Generative Models such as Variational AutoEncoder VAE, and Generative Adversarial Networks GANs
- Attentions-based learning
- Self-supervised and semi-supervised learning
- Large Protein Models.

These models will be instrumental in advancing our understanding of T cell biology and developing models capable of solving immunological challenges.

Training Opportunities

Our collaborative research spans expertise across multiple disciplines, including mathematical and statical modelling, machine-learning and artificial intelligence AI applied to immunological data, data science, and T cell and tissue immunology.

Depending on their research interests and chosen direction within the group, the candidate will receive tailored supervision and mentorship to support their academic and professional development.

In additions to guidance from our team, the University of Oxford provides world-class teaching and supervisory resources, which will be available to the candidate. The candidate will also have opportunities to participate in conferences, workshops, and other academic events to facilitate networking, skill transfer and further professional growth.

Key Publications

1) M. B. D. Hudson; R. A. Ricardo, G. Ogg, H. Koohy, Can we predict T cell specificity with digital biology and machine learning? *Nature Reviews Immunology*, (2023).
2) B. McMaster, C. Thorpe, G. Ogg, C. M. Deane, H. Koohy, Can AlphaFold's breakthrough in protein structure help decode the fundamental principles of adaptive cellular immunity? *Nat Methods* 21, 766-776 (2024).

3) W. L. e. al, Disease associated human TCR characterization by deep-learning framework TCR-DeepInsight. *bioRxiv*, (2023).
4) L. Wu *et al.*, huARdb: human Antigen Receptor database for interactive clonotype-transcriptome analysis at the single-cell level. *Nucleic Acids Res* 50, D1244-D1254 (2022).
5) C. H. Lee *et al.*, A robust deep learning workflow to predict CD8 + T-cell epitopes. *Genome Med* 15, 70 (2023).
6) D. Corridoni *et al.*, Single-cell atlas of colonic CD8(+) T cells in ulcerative colitis. *Nat Med* 26, 1480-1490 (2020).

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5.2 Decoding the Underlying Principles of T Cell Receptor Recognition of CD1a/Lipid-Antigens by Developing Integrative Machine Learning Models

Prof. Hashem Koohy & Prof. Graham Ogg

Contact Email Address:

hashem.koohy@rdm.ox.ac.uk;

graham.ogg@ndm.ox.ac.uk

Project Overview

T cell immunity is a critical component of the adaptive immune system. Antigen-specific T cell responses are initiated when the T cell receptor (TCR) interacts with an antigen, typically presented as a peptide by the major histocompatibility complex (MHC) on the surface of target cells.

While much of our understanding of T cell interactions comes from peptide-based antigens presented by MHC molecules(*1, 2*), recent research has identified a subpopulation of T cells with TCRs that recognize lipid antigens presented by CD1 molecules. These lipid-specific T cells have significant therapeutic potential and play a role in driving immunopathological diseases(*3*). However, there is a substantial knowledge gap regarding the mechanisms of lipid-specific TCR recognition of lipids displayed by CD1 isoforms. This project aims to develop computational and machine learning models to elucidate the principles underlying lipid presentation by CD1, and lipid-specific T cell recognition.

Background

Over the past few decades, computational models for predicting peptide antigen presentation, such as netMHCpan, have been developed with remarkable success. These models, particularly for MHC class I peptides, have been instrumental in predicting peptide-MHC binding and advancing vaccine development and cancer immunotherapy. Despite the success of peptide prediction models, there are no equivalent models for predicting lipid ligands for CD1 molecules.

In parallel, cutting-edge single-cell technologies have emerged, generating vast amounts of data, including TCR sequences, transcriptomics, and proteomics. These advances have led to significant progress in understanding conventional TCR-antigen interactions, but little effort has been made to study CD1-lipid-specific T cell interactions due to the lack of data. As a result, lipid T cell recognition of lipid complexes (CD1) remain an underexplored area. This project will focus on CD1a-reactive T cells from human skin inflammatory diseases and aims to address this gap by developing integrative machine learning models to predict CD1a-specific TCR interactions with CD1a molecules.

Project Objectives

Building on our ongoing research, this collaborative project aims to leverage mass spectrometry MS and single-cell data and the power of AI and deep neural networks (DNNs) to decode the fundamental principles of CD1-reactive T cell immunity. It therefore aims to cover two primary objectives:

1) Develop machine learning models using recently published CD1 ligandome data to identify features associated with lipid presentation by CD1 isoforms.

2) Computationally identify features that govern CD1a-lipid recognition by CD1a-reactive T cells.

Methodology

The project will utilize CD1 lipidomic MS data from a recently published study(*4*), applying Bayesian statistical models to identify parameters associated with lipid presentation by the four CD1 isoforms. The goal is to develop a predictive model for CD1 lipid ligands. Additionally, multiomics single-cell data from in-house CD1a-specific T cells (both stimulated and unstimulated) will be used to explore features of TCR recognition of CD1a. These insights will help build machine learning models to map TCRs to their cognate CD1a ligands.

While some T cells use conserved TCRs to recognize nonpolymorphic antigen-presenting molecules CD1d,b and MR1 by Natural Killer T cells, germline-encoded mycolyl-reactive T cells and Mucosal-associated invariant T cells respectively, motifs associated with CD1a-reactive T cells are largely unknown and under investigation. We will be building on top of ongoing research (*5*) by using in-house and public CD1a-reactive TCR datasets and implementing advanced ML methods to identify enriched motifs.

Applications and Impact

This project represents a multidisciplinary approach to addressing a critical gap in our understanding of unconventional T cell immunity. By leveraging integrative machine learning models and CD1a-specific TCR data, this research will contribute to a deeper understanding of skin inflammatory disease progression and the development of therapeutic interventions.

Disease Relevance

In the final stage of this project, we will apply the developed models to address critical immunological questions related to inflammatory skin diseases such as atopic dermatitis (AD) and psoriasis (PS). This project will provide deeper insights into the development and progression of these inflammatory skin disorders. The data will be integrated with in-house and public tissue TCR data before and after therapeutic intervention, in order to define relevance of T cell subsets with disease. More specifically, it will allow us to more systematically identify factors associated with T cell self-reactivity and autoimmune responses. Ultimately, this research aims to contribute to the development of preventive and/or therapeutic strategies for these debilitating conditions.

Key Technology

The data utilized in our research are typically sourced from public repositories or generated through our Oxford work including collaborations with experimental partners.

These datasets are primarily derived from mass spectrometry MS techniques and from cutting-edge single cell and spatial transcriptomics sequencing, as well as advanced imaging technologies. They encompass a wide range of molecular readouts, including paired alpha-beta TCR sequencing, transcriptomic profiles, and proteomics. For some T cells with known antigen-specificity, information about their corresponding antigens is also available.

The candidate will have the opportunity to gain hands-on experience with these emerging state-of-the-art single cell technologies and the rich dataset they generate.

From a model development perspective, these large, high-dimensional datasets offer an excellent platform for leveraging groundbreaking AI/ML and Protein Language Models PLM. Prominent approaches include, but are not limited to:

- Deep Generative Models such as Variational AutoEncoder VAE, and Generative Adversarial Networks GANs
- Attentions-based learning
- Self-supervised and semi-supervised learning
- Large Protein Models.
- Use of protein structure prediction models
- Use of AI models for prediction of Protein-Protein Interaction (PPI)
- Statistical Bayesian Models

These models will be instrumental in advancing our understanding of T cell biology and developing models capable of solving immunological challenges.

In summary, the project will involve a comprehensive literature review, data curation, and alignment to create high-quality datasets. The candidate will develop skills in data science, computational modelling and immunology. Advanced AI techniques, including deep generative models, attention-based learning, and self- or semi-supervised learning, will be applied to generate interpretable low-dimensional latent spaces for downstream tasks like mathematical modelling and synthetic data generation.

Training Opportunities

Our collaborative research spans expertise across multiple disciplines, including T cell and structural immunology, dedicated mathematical and statistical modelling, machine-learning and artificial intelligence AI applied to immunological data and data science.

Depending on their research interests and chosen direction within the group, the candidate will receive tailored supervision and mentorship to support their academic and professional development.

In additions to guidance from our team, the University of Oxford provides world-class teaching and supervisory resources, which will be available to the candidate. The candidate will also have opportunities to participate in conferences, workshops, and other academic events to facilitate networking, skill transfer and further professional growth.

Key Publications

1) M. B. D. Hudson; R. A. Ricardo, G. Ogg, H. Koohy, Can we predict T cell specificity with digital biology and machine learning? Nature Reviews Immunology, (2023).
2) B. McMaster, C. Thorpe, G. Ogg, C. M. Deane, H. Koohy, Can AlphaFold's breakthrough in protein structure help decode the fundamental principles of adaptive cellular immunity? Nat Methods 21, 766-776 (2024).
3) Y. L. Chen et al., Group A Streptococcus induces CD1a-autoreactive T cells and promotes psoriatic inflammation. Sci Immunol 8, eadd9232 (2023).
4) S. Huang et al., CD1 lipidomes reveal lipid-binding motifs and size-based antigen-display mechanisms. Cell 186, 4583-4596 e4513 (2023).
5) E. Bryan et al., Human Skin T Cells Express Conserved T-Cell Receptors that Cross-React with Staphylococcal Superantigens and CD1a. J Invest Dermatol 144, 833-843 e833 (2024).

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5.3 The TIGER project V2.0

Prof. Paul Klenerman & Dr. Nick Provine

Contact Email Address:

paul.klenerman@ndm.ox.ac.uk;

nicholas.provine@ndm.ox.ac.uk

Project Overview

TIGER (T cells - Innate-like, Gut-Enriched and Resident) is a project to discover new innate-like T cells in human tissues and to define new functions of the cells we have already discovered. We originally identified CD161+ T cell populations in the liver (eg MAIT cells and Vd2 cells) which have an overlapping set of properties - commensal reactive, innate-like (responding to IL12 and 18) and enriched in tissue. These have a combination of a distinct phenotype and a distinct specificity, associated with a distinct TCR usage. Such cells are involved in normal host defence and possess a range of properties which include tissue repair as well as host defence, including against viruses. Recently we have also explored how they are involved in early responses to vaccines. Given the abundance of these populations in humans, we believe there are more cell types out there to be discovered which could be important in health and disease. We also are beginning to recognise the brand range of effector functions they can exert in tissues.

In TIGER v1.0 we aimed to follow up on new populations of T cells from the human gut and other tissues and demonstrate that they are:

1) Commensal specific
2) Define the restricting allele(s) which may include novel molecules.
3) Define the ligand.

Using this approach we identified a new set of CD4+ T cells we called Tmics (see https://www.nature.com/articles/s41467-022-35126-3)

In TIGER v2.0:

1) We will address to what extent these Tmic as well as other unconventional T cells are involved in disease by examining samples from patients with inflammatory bowel disease, celiac disease and liver inflammation and address their modulation in response to infections such as influenza and SARS-CoV-2. We will also use novel CRISPR based screens (CHIME) to explore the origin of these cells and their development and maintenance in vivo.
2) We will continue to explore how unconventional cell types and early/innate signals are involved in priming and sustaining responses to different vaccines using human samples and mechanistic models.
3) We will work with colleagues at the MORU and OUCRU sites in SE Asia to analyse how these cell populations respond to infectious diseases of global significance (eg TB, dengue). This work is in the context of larger initiatives with these colleagues such as the SEACOVARIANTs programme.
4) We will explore how these unconventional T cell populations vary across the animal kingdom in collaboration with groups from Pirbright, Roslin and ZSL.

A given project could focus on any of these aims, alone or in combination. A recent paper in this area using. range of the tools is

Garner, L.C., Amini, A., FitzPatrick, M.E.B. *et al.* Single-cell analysis of human MAIT cell transcriptional, functional and clonal diversity. *Nat Immunol* (2023). https://doi.org/10.1038/s41590-023-01575-1

Disease Relevance

This project is relevant to

1) Vaccine development for infectious diseases
2) Natureal immunity against sever viral infection such as influenza
3) Inflammatpry disease in the gut and liver：Development of immune responses to cancers and following checkpoint therapy

Key Technology

We apply a number of techniques which in our lab typically include:

T cell immunology, FACS, FACS sorting, single cell genomics, spatial transcriptomics. CRISPR screens and functional screens will be used to hone in on the populations of interest. This will include T cell cloning, associated with sequencing, transfection and generation of reagents to address the specificity of particular populations.

Training Opportunities

- The student will be trained in murine handling and experimentation according to strict UK Home Office welfare standards
- The student will be trainined in cellular immunology, flow cytometry, single cell RNA and ATAC sequencing; library prep and sequencing analysis.
- The student will be trained in bioinformatic analyses of the datasets generated

Prospective students are encouraged to contact Paul Klenerman directly to discuss specific details of this project to find out more about the precise research questions which most interest them.

Key Publications

1) van Wilgenburg, B., L. Loh, Z. Chen, T.J. Pediongco, H. Wang, M. Shi, Z. Zhao, M. Koutsakos, S. Nussing, S. Sant, Z. Wang, C. D'Souza, X. Jia, C.F. Almeida, L. Kostenko, S.B.G. Eckle, B.S. Meehan, A. Kallies, D.I. Godfrey, P.C. Reading, A.J. Corbett, J. McCluskey, **P. Klenerman***, K. Kedzierska, and T.S.C. Hinks, *MAIT cells contribute to protection against lethal influenza infection in vivo.* Nat Commun, 2018. **9**(1): p. 4706. (195 citations)
2) Cupovic, J., S.S. Ring, L. Onder, J.M. Colston, M. Lutge, H.W. Cheng, A. De Martin, N.M. Provine, L. Flatz, A. Oxenius, E. Scandella, P. Krebs, D. Engeler, **P. Klenerman***, and B. Ludewig, *Adenovirus vector vaccination reprograms pulmonary fibroblastic niches to support protective inflating memory CD8(+) T cells.* Nat Immunol, 2021. **22**(8): p. 1042-1051.
3) Provine, N.M., A. Amini, L.C. Garner, A.J. Spencer, C. Dold, C. Hutchings, L. Silva Reyes, M.E.B. FitzPatrick, S. Chinnakannan, B. Oguti, M. Raymond, M. Ulaszewska, F. Troise, H. Sharpe, S.B. Morgan, T.S.C. Hinks, T. Lambe, S. Capone, A. Folgori, E. Barnes, C.S.

Rollier, A.J. Pollard, and **P. Klenerman**, *MAIT cell activation augments adenovirus vector vaccine immunogenicity.* Science, 2021. **371**(6528): p. 521-526.

4) Hackstein, C.P., D. Costigan, L. Drexhage, C. Pearson, S. Bullers, N. Ilott, H.D. Akther, Y. Gu, M.E.B. FitzPatrick, O.J. Harrison, L.C. Garner, E.H. Mann, S. Pandey, M. Friedrich, N.M. Provine, H.H. Uhlig, E. Marchi, F. Powrie, **P. Klenerman***, and E.E. Thornton, *A conserved population of MHC II-restricted, innate-like, commensal-reactive T cells in the gut of humans and mice.* Nat Commun, 2022. **13**(1): p. 7472.
5) Garner, L.C., A. Amini, M.E.B. FitzPatrick, M.J. Lett, G.F. Hess, M. Filipowicz-Sinnreich, N.M. Provine, and **P. Klenerman**, *Single-cell analysis of human MAIT cell transcriptional, functional and clonal diversity.* Nature Immunology, 2023. **24** (9) 15-65-78.

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5.4 Discovering New Post-Translational Mechanisms Controlling T-cell Immunity across scales, from tissues to single proteins

Dr. Pablo F. Céspedes

Contact Email Address:

pablo.cespedes@ndm.ox.ac.uk

Project Overview

Although the human genome encodes more than 20,000 protein-coding genes, human cells enhance protein diversity through the regulation of RNA transcription initiation and termination, as well as the splicing of messenger RNAs (mRNAs). These mRNAs are then used as blueprints for protein synthesis in a process known as mRNA translation. Proteins, however, are far from static entities; they undergo additional chemical modifications to participate in the dynamic processes that sustain life. These modifications, commonly referred to as post-translational modifications (PTMs), alter protein trafficking, charge, topology, and affinity for other molecules, such as lipids, sugars, and nucleic acids.

In the last decade, we have gained significant insights into the crosstalk between bodily systems, highlighting the central role of the immune system in coordinating the functions of the endocrine, nervous, cardiovascular, integumentary (skin), and digestive systems. Additionally, immune cells communicate with trillions of microbes residing in the integumentary and digestive systems, distinguishing between foes and symbiotic partners.

Notably, most host proteins involved in this multisystem communication are post-translationally modified. However, our understanding of how PTMs regulate immune processes remains limited. This is partly because human evolution has selected for a unique combination of enzymes responsible for these modifications, which are poorly replicated by experimental models such as rodents and non-human primates.

As part of our group's long-term mission to discover key human enzymes that regulate immune receptor functionality, we have developed a discovery framework utilizing the latest advancements in semi-synthetic biology and ex vivo 3Rs models. These technologies include synthetic antigen-presenting cells (sAPCs), lymphoid organoids, and lymphoid explants, which enable us to study T-cell communication at various time and length scales—from nanometres to millimetres, and from milliseconds to weeks. Our group has already begun investigating human-specific PTMs in the context of:

1) Modifications of key peptidyl arginines critical for cell-cell communication.
2) Sugars shaping receptor-ligand interactions at the T-cell glycocalyx.

We have conducted CRISPR/Cas9 screens and identified novel PTM enzymes that modulate T-cell communication. Your role as part of the team will be to characterize the substrates and processes being modulated across molecular and tissue scales. We will then assess the functional relevance of these PTM enzymes in human disease by mining publicly available datasets. Through this work, we aim to identify novel mechanisms that regulate lymphocyte function and develop new therapeutic strategies.

Disease Relevance

- The funcional relevance of these enzymes will be tested as part of mechanistic studies elucidating homeostatic mechanisms and the pathogenesis of autoimmunity, infection, inflammation, aging, metabolic dysregulation and cancer.
- The Cespedes group collaborates with many other groups under the COI umbrella, so the applications of our knowledge base and methodological framework will not be limited to mechanistic studies and can be dynamically shaped to the working hypothesis of the project.
- Potential collaborations include: studying the effector mechanisms of T cell clones expressing different integrins and C-type lectins in cancer (Professor Tao Dong), inflammatory bowel disease (Dr Matthias Friedrich Lab), modelling tumor metastases, vaccinology (Dr Jack Tan, Dr Pramila Rijal) and infectious diseases (Dr Peter Wing), spatial transcriptomics for niche and pathway distribution in response to organoid and explant manipulations (Dr Matthew Bottomley), pathogenesis of malignant pleural effusions and pleural infections (Dr. Nikolaos Kanelakis), Metabolomics (Dr Adan Pinto-Fernandes).
- Potential collaborations in other areas of molecular immunology and biophysics including the study of forces regulating T-cell activation (Professor Marco Fritzsche), Protein engineering (Dr Ricardo Fernandes), and the development of new methods to study the physical communication of cells (Professor Michael L. Dustin).
- Combined with our CRISPR/Cas9, high-dimensional and high-througput microscopy approaches, the candidate will gain substantial training in cutting-edge molecular, cellular and tissue-level immunological methods. Acquiring a future-proof skillset.

Key Technology

- Compresstome for precision cut lymphoid tissue slices.
- Human lymphoid organoids.
- CRISPR/Cas9 knock-outs and knock-ins.
- Spectral flow cytometry.
- Incucyte SX5 live imaging system.
- Super-resolution microscopy.
- Super-resolution flow cytometry.
- Single-cell RNA sequencing.
- High-mass resolution DDA and DIA LC-MS/MS.
- Click chemistry.
- Metabolomics.
- Mouse experiments to model multisystemic mechanisms of immune regulation.

Training Opportunities

My goal is to be a mentor who provides opportunities to develop a future-proof skill set, grounded in rigorous scientific reasoning and the ability to channel creativity into focused efforts. I aim to strengthen your skills by fostering a dynamic learning environment that nurtures curiosity, self-awareness, and professional growth.

No prior experience is required, as candidates will be trained in cutting-edge molecular, cellular, and tissue-level immunology techniques. In addition, support will be provided for mastering informatics pipelines relevant to statistics and the analysis of complex transcriptomics and proteomics datasets.

Through collaborations, students will gain exposure to fundamental fields intersecting with immunology, such as biochemistry, biophysics, genetic engineering, protein engineering, virology, computational biology, and statistics.

Key Publications

1) Hamid, M.H.B.A., Cespedes, P.F., Jin, C. et al. Unconventional human CD61 pairing with CD103 promotes TCR signaling and antigen-specific T cell cytotoxicity. Nat Immunol 25, 834–846 (2024). https://doi.org/10.1038/s41590-024-01802-3
2) Céspedes, P.F., Jainarayanan, A., Fernández-Messina, L. et al. T-cell trans-synaptic vesicles are distinct and carry greater effector content than constitutive extracellular vesicles. Nat Commun 13, 3460 (2022). https://doi.org/10.1038/s41467-022-31160-3
3) Schneider, F., Cespedes, P.F., Karedla, N. *et al.* Quantifying biomolecular organisation in membranes with brightness-transit statistics. *Nat Commun* **15**, 7082 (2024). https://doi.org/10.1038/s41467-024-51435-1
4) David G Saliba, Pablo F Céspedes-Donoso, Štefan Bálint, Ewoud B Compeer, Kseniya Korobchevskaya, Salvatore Valvo, Viveka Mayya, Audun Kvalvaag, Yanchun Peng, Tao Dong, Maria-Laura Tognoli, Eric O'Neill, Sarah Bonham, Roman Fischer, Benedikt M Kessler, Michael L Dustin (2019) Composition and structure of synaptic ectosomes exporting antigen receptor linked to functional CD40 ligand from helper T cells eLife 8:e47528. https://doi.org/10.7554/eLife.47528

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6. Structure Biology

6.1 Study SARS-CoV-2 infection in situ by CryoET

Prof. Peijun Zhang

Contact Email Address:

peijun.zhang@strubi.ox.ac.uk

Project Overview

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

Disease Relevance

SARS-CoV-2 infection, Vaccines

Key Technology

- cryoEM single particle analysis
- cryo-electron tomography
- in situ structural biology
- cryoFIB/SEM lamella preparation
- cryoFIB/SEM volume imaging
- Correlative fleorescence and electron microscoptSuper-resolution microscopy.

Training Opportunities

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

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

Key Publications

1) Mendonça L, Howe A, Gilchrist JB, Sheng Y, Sun D, Knight ML, Zanetti-Domingues LC, Bateman B, Krebs AS, Chen L, Radecke J, Li VD, Ni T, Kounatidis I, Koronfel MA, Szynkiewicz M, Harkiolaki M, Martin-Fernandez ML, James W, Zhang P* (2021) Correlative multi-scale cryo-imaging unveils SARS-CoV-2 assembly and egress. Nat Commun. 12(1):4629
2) Ni T, Mendonça L, Zhu Y, Howe A, Radecke J, Shah PM, Sheng Y, Krebs AS, Duyvesteyn HME, Allen E, Lambe T, Bisset C, Spencer A, Morris S, Stuart DI, Gilbert S, Zhang P* (2023) ChAdOx1 COVID vaccines express RBD open prefusion SARS-CoV-2 spikes on the cell surface. Iscience 26 (10).
3) Ni T, Zhu Y, Yang Z, Xu C, Chaban Y, Nesterova T, Ning J, Böcking T, Parker MW, Monnie C, Ahn J, Perilla JR, Zhang P* (2021) Structure of native HIV-1 cores and their interactions with IP6 and CypA. Sci Adv 7(47):eabj5715.
4) Ni T, Gerard S, Zhao G, Dent K, Ning J, Zhou J, Shi J, Anderson-Daniels J, Li W, Jang S, Engelman AN, Aiken C, Zhang P* (2020) Intrinsic curvature of HIV-1 CA hexamer underlies capsid topology and interaction with cyclophilin A. Nat Struct Mol Biol 27, 855–862.
5) Zhao G., Perilla J.R., Yufenyuy E.L., Meng X., Chen B., Ning J., Ahn J., Gronenborn A.M., Schulten K.*, Aiken C.* and Zhang P.* (2013) Mature HIV-1 Capsid Structure by Cryo-electron Microscopy and All-atom Molecular Dynamics. Nature 497(7451):643-6

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6.2 HIV-1 nuclear transport and host genome integration

Prof. Peijun Zhang

Contact Email Address:

peijun.zhang@strubi.ox.ac.uk

Project Overview

Human immunodeficiency virus type 1 (HIV-1) is the causative agent behind acquired immunodeficiency syndrome (AIDS) that currently has no cure or vaccine. While antiviral treatments are effective, the rise of drug-resistant strains has become a growing concern. HIV-1 primarily infects the immune system, targeting CD4+ T cells and macrophages and is a lentivirus known to be able to infect non-dividing cells, requiring it to exploit nuclear transport mechanisms. This process is dependent on the viral capsid. The HIV capsid is a conical structure that contains the genomic material of the virus, and protect it from host innate immunity. How does the capsid navigate through the nucleus and arrive at the site of host chromatin for integration? When and where does the capsid uncoate to release its viral genome? What triggers capsid uncoating? These are key questions to understand HIV-1 infection, in particular in the cell nucleus.

We aim to apply multi-imaging modalities to investigate uncoating and nuclear transport of HIV-1. These will include super-resolution fluorescence microscopy (including the newest MINFLUX system), Focused Ion Beam and Scanning electron microscopy (cryoFIB/SEM), cryo-electron microscopy and cryo-electron tomography (cryoEM/ET). The viral core and host factors will be fluorescently tagged using non-natural AA and click chemistry and infection will be monitored from viral attachment to nuclear import. The sample will be cryo-preserved and imaged by cryoEM/ET and cryoFIB/SEM. The combination of these imaging techniques will yield unparalleled structural information of the HIV infection process within the native cells, providing the framework for development of novel therapeutics targeting HIV infection in the future.

Disease Relevance

HIV-1, retrovirus infection

Key Technology

- cryoEM single particle analysis
- cryo-electron tomography
- in situ structural biology
- cryoFIB/SEM lamella preparation
- Correlative fleorescence and electron microscopt

Training Opportunities

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

techniques in molecular, cellular and structural biology. Through the projects, students will be trained in

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

Key Publications

1) Hou Z, Nightingale F, Zhu Y, MacGregor-Chatwin C, Zhang P* (2021) Structure of native chromatin fibres revealed by Cryo-ET in situ. Nature Communications 14 (1), 6324
2) Krebs AS, Liu HF, Zhou Y, Rey JS, Levintov L, Shen J, Howe A, Perilla JR, Bartesaghi A, Zhang P* (2023) Molecular architecture and conservation of an immature human endogenous retrovirus. Nat Commun 14(1):5149
3) Ni T, Zhu Y, Yang Z, Xu C, Chaban Y, Nesterova T, Ning J, Böcking T, Parker MW, Monnie C, Ahn J, Perilla JR, Zhang P* (2021) Structure of native HIV-1 cores and their interactions with IP6 and CypA. Sci Adv 7(47):eabj5715
4) Ni T, Gerard S, Zhao G, Dent K, Ning J, Zhou J, Shi J, Anderson-Daniels J, Li W, Jang S, Engelman AN, Aiken C, Zhang P* (2020) Intrinsic curvature of HIV-1 CA hexamer underlies capsid topology and interaction with cyclophilin A. Nat Struct Mol Biol 27, 855–862
5) Zhao G., Perilla J.R., Yufenyuy E.L., Meng X., Chen B., Ning J., Ahn J., Gronenborn A.M., Schulten K.*, Aiken C.* and Zhang P.* (2013) Mature HIV-1 Capsid Structure by Cryo-electron Microscopy and All-atom Molecular Dynamics. Nature 497(7451):643-6

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6.3 Structural studies in human muscle nAChR

Dr. Yin Dong & Dr. David Sauer

Contact Email Address:

yin.dong@ndcn.ox.ac.uk;

david.sauer@cmd.ox.ac.uk

Project Overview

Background

Muscle nicotinic acetylcholine receptors (nAChR) are the essential ligand gated ion channel that receive the acetylcholine neurotransmitter from motor neurons to initiate skeletal muscle membrane depolarisation and the subsequent muscle contraction. This is essential to all voluntary movements. Disruption of this, either by autoimmune antibodies or mutations in the genes encoding the receptor subunits, leads to myasthenic syndromes (myasthenia gravis or congenital myasthenia respectively). Muscle nAChR is the most complex member of the pentameric ligand gated ion channel (pLGIC) family, which also includes other neurotransmitter receptors such as serotonin receptors and GABAA receptors, composed of 2 α1 subunits, 1β1 subunit, a δ, and either a γ subunit in the foetal receptor, or an ε subunit in the adult form. This family of receptors can rapidly transition between multiple functional states upon ligand binding, translating chemical signals into electrical signals, enabling the subtle changes in depolarisation frequency that is essential to neuronal activity and fine motor control.

Muscle nAChR is the target of numerous naturally occurring toxins, such as snake venom and curare, and drugs such as neuromuscular blocking agents and fluoxetine. Therefore, there is considerable interest in understanding where and how the drugs bind to enable further drug discovery. The complexity of muscle nAChR has made it challenging to produce in the quantities needed for structural studies, with most previous studies carried out on the homologous electric organ receptors from electric rays.

Previous work

We have previously obtained initial structures of the adult receptor using a recombinant expression system, and a custom purification protocol. We have also identified a novel positive allosteric modulator (PAM) that is selective for adult muscle nAChR. Development of this compound into more drug-like molecules would be greatly aided by structural information on where it binds to the receptor to enable structure-based drug design.

Aim

The aim of this project is to obtain high resolution cryo-EM structures of human muscle nAChR in different conformations, and in complex with a PAM molecule.

Project plan

Using a high through Ca^{2+} FLUX assay, different combinations of mutations and pharmacological compounds will be screened to identify combinations that increase nAChR activity, and preferentially adopt the open conformation. These will be confirmed by single channel electrophysiology. Stable cell lines will then be created of mutant channels using an established lentiviral expression system. Protein purification and cryo-EM sample preparations will then be initiated using previously developed methods, but may require further optimisation – such as testing different lipids in nanodisc reconstitution, and other membrane mimetic systems such as liposomes. Cryo-EM data collection and analysis, will be carried out using existing software and workflows.

Once structures are obtained, further functional characterisations will be carried out to test hypotheses that arise from the structural information. These can be carried out by single channel electrophysiology or Ca^{2+} FLUX assays.

Disease Relevance

Myasthenia gravis (MG) is the most common form of neuromuscular disease, affecting ~150 per million people. 90% of cases are caused by antibodies against the muscle type nAChR. Congenital myasthenic syndromes (CMS) affects around 9 per million children in the UK. The most common cause of CMS is mutations in genes that code for the different subunits of nAChR, affecting roughly half of all patients. Both MG and CMS cause fatigable muscle weakness, and can affect all voluntary muscles, including respiratory and bulbar muscles, which in severe cases can lead to death. Treatments to both conditions are limited, particularly for congenital myasthenic syndromes, where only symptomatic treatments are available, leaving most patients with some sort of disability even on optimised treatment

One of the most severe subtypes of CMS is fast channel syndrome, where mutations cause abnormally brief channel openings. Fast channel patients tend to respond poorly to all current treatments, often causing premature death. We have demonstrated that our PAM increases the channel openings of fast channel mutants to WT levels, potentially offering a disease altering treatment for these patients. A PAM for nAChR that boosts NMJ signalling will also likely be beneficial for other subtypes of CMS and MG, as inadequate NMJ signalling is the primary cause of disease in most subtypes.

Key Technology

The key technologies used in this project will be single particle cryo-electron microscopy, possibly cryo-electron tomography, single channel electrophysiology, calcium FLUX assays, and stable cell line generation using lentiviral technology. Access to all the key technologies is well-established by the host laboratories.

Training Opportunities

Training will be available from experienced post-doctoral scientists in all aspects of this project. There will be opportunities to learn how to express and purify a very challenging heteromultimeric human membrane protein, and determine its structure using cryo-electron microscopy.

More specifically, the student will learn how to culture suspension adapted mammalian cells, chemically transfect them, generate stable cell lines using lentivirus, and isolate clones that express AChR using FACS. They will purify the protein by isolating cell membranes via cell fractionation, affinity purification and size exclusion chromatography. They will learn to reconstitute nAChR into membrane mimetic systems such as lipidic nanodiscs, preparing samples for cryo-electron microscopy, data collection and data analysis.

The student will also have opportunities to learn how to assess nAChR function using a cell based Ca2+ flux assay, and single channel electrophysiology.

Key Publications

1) Dong YY*, Wang H*, Pike ACW*, 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. “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 2015 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. “The structural basis of ZMPSTE24-dependent laminopathies” Science 2013 339(6127):1604-7 * Joint 1st authors
4) David B. Sauer, Jinmei Song, Bing Wang, Jacob K. Hilton, Nathan K. Karpowich, Joseph A. Mindell, William J. Rice & Da-Neng Wang. “Structure and inhibition mechanism of the human citrate transporter NaCT” Nature 2021 591, pages157–161
5) Huanyu Z Li, Ashley CW Pike, Irina Lotsaris, Gamma Chi, Jesper S Hansen, Sarah C Lee, Karin EJ Rödström, Simon R Bushell, David Speedman, Adam Evans, Dong Wang, Didi He, Leela Shrestha, Chady Nasrallah, Nicola A Burgess-Brown, Robert J Vandenberg, Timothy R Dafforn, Elisabeth P Carpenter, David B Sauer “Structure and function of the SIT1 proline transporter in complex with the COVID-19 receptor ACE2” Nature Communications 2024 15, Article number: 5503

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6.4 Characterizing the ultrastructure of coronavirus replication complexes using in-situ cryo-Electron Tomography

Dr. Loic Carrique, Dr. Peter Wing & Prof. Johnathan Grimes

Contact Email Address:

loic.carrique@strubi.ox.ac.uk;

peter.wing@ndm.ox.ac.uk;

jonathan.grimes@strubi.ox.ac.uk

Project Overview

SARS-CoV-2 marked the third zoonotic insertion of a pandemic coronaviral infection into the human population, causing one of the greatest global health challenges to date. As a member of the Coronaviridae family, SARS-CoV-2 is an enveloped virus with a positive non-segmented RNA genome. Viral entry is mediated through the interaction of Spike with ACE2 at the cell surface that triggers the fusion of the viral envelope with the cellular membrane. The viral RNA is released into the cytoplasm where translation of the non-structural proteins from the viral RNA genome induces the formation of the viral replicase complex consisting of interconnected membranous compartments referred to as double-membrane vesicles (DMVs). Assembly of nascent virions occurs in these modified cellular structures derived from components of the endoplasmic reticulum and Golgi complex and new particles egress from assembly structures via exocytosis. A key gap in our knowledge is whether there are distinctions in how different coronaviruses manipulate the cellular ultrastructure to establish replication complexes and productive infection.

Technical advancements in cryo-electron microscopy (cryo-EM) have facilitated high-resolution visualization of macromolecules and pathogens in their native biological settings, including cellular organelles, whole cells, and tissues. Due to limited electron penetration, cryo-focused ion beam combined with scanning electron microscopy (cryo-FIB/SEM) was developed to create 200 nm thick lamellae for high-resolution cryo-electron tomography (cryo-ET). Correlative light and electron microscopy (CLEM) is essential for identifying and targeting regions of interest for cryo-FIB milling and cryo-ET data collection. Cryo-EM imaging of the SARS-CoV-2 life cycle has revealed spatially organized viral replication within dedicated cytoplasmic compartments. Over the past decade, the Oxford Particles Imaging Centre (OPIC) has established a comprehensive pipeline, from sample vitrification to high-resolution cryo-EM and cryo-ET data collection, specializing in live CL3 pathogens, a unique capability in Europe. The Division of Structural Biology has a long history of viral research, including studies on Influenza, RSV, Hantan, Nipah, Dengue, SARS-CoV-2, Rotaviruses, Hepatitis A, B, C & D, EV71, and Noroviruses.

The aim of this project is to explore the fundamental aspects of sub-cellular compartmentalisation of coronaviral infection focusing on the structural composition of the replicase complex. Using the world-leading cryo-EM bio-imaging capabilities of OPIC, this project will answer essential questions surrounding the formation, structure, and spatial organisation of viral replication complexes in a variety of cellular conditions. Recent work in the Wing lab has shown a dynamic interplay between cellular oxygen sensing and coronaviral replication yet, how this affects the formation of replication complexes remains unclear. While this work has shed light on the dynamic relationship between these processes, significant questions remain unanswered. Specifically, the impact of this interaction on the formation of

viral replication complexes requires deeper exploration. Future studies will focus on elucidating the mechanisms by which oxygen sensing pathways influence the assembly and function of these complexes. Such insights could potentially reveal novel targets for antiviral therapies and contribute to our understanding of coronaviral pathogenesis.

Disease Relevance

Coronaviruses have emerged as significant pathogens with substantial disease relevance in recent years. These RNA viruses are known for their ability to cause respiratory illnesses in humans and animals, ranging from mild common colds to severe acute respiratory syndromes. The disease relevance of coronaviruses became particularly evident with the emergence of SARS-CoV in 2002, MERS-CoV in 2012, and most notably, SARS-CoV-2 in 2019, which caused the global COVID-19 pandemic. These outbreaks have demonstrated the potential of coronaviruses to cause widespread morbidity and mortality, overwhelm healthcare systems, and disrupt global economies. The zoonotic nature of coronaviruses, allowing them to jump from animal reservoirs to humans, further underscores their importance in public health. Additionally, the ability of coronaviruses to mutate rapidly and potentially evade immune responses poses ongoing challenges for vaccine development and therapeutic interventions, highlighting the critical need for continued research and surveillance in this field.

Key Technology

The key techniques involved in this project include cryo-electron microscopy (cryo-EM), tomography, and molecular virology. Advanced imaging techniques will be employed at the Oxford Particle Imaging Centre (OPIC) to investigate the structural composition and spatial organization of coronaviral replication complexes. Cryo-EM allows for high-resolution visualization of biological structures in their native state, making it an ideal tool for studying the subcellular compartmentalization of viral infection and the formation of replication complexes under various cellular conditions.

Training Opportunities

This project offers training opportunities in:

1) Advanced cryo-electron microscopy techniques at the Oxford Particle Imaging Centre (OPIC)
2) Studying subcellular compartmentalization of coronaviral infection
3) Analysing sthe structural composition of viral replicase complexes
4) Investigating the formation, structure, and spatial organization of viral replication complexes under various cellular conditions
5) Exploring the relationship between cellular oxygen sensing and coronaviral replication
6) Examining the impact of oxygen sensing pathways on the assembly and function of viral replication complexes
7) Developing skills in elucidating mechanisms of viral pathogenesis
8) Potential involvement in identifying novel targets for antiviral therapies

These opportunities will provide hands-on experience with cutting-edge bio-imaging technologies and contribute to the understanding of coronaviral infection processes.

Key Publications

1) Georg Wolff *et al.* A molecular pore spans the double membrane of the coronavirus replication organelle. *Science* (2020).
2) Wing, P.A.C.*, et al.* Hypoxic and pharmacological activation of HIFs inhibits SARS-CoV-2 infection of lung epithelial cells. *Cell Reports* (2021).
3) Staller, E., Carrique, L., Swann, O.C. *et al.* Structures of H5N1 influenza polymerase with ANP32B reveal mechanisms of genome replication and host adaptation. *Nat Commun* 15, 4123 (2024).
4) Huang, Y., Wang, T., Zhong, L. *et al.* Molecular architecture of coronavirus double-membrane vesicle pore complex. *Nature* 633, 224–231 (2024).

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7. Other

7.1 Integrating omics for accurate diabetes diagnosis and stratification

Dr. Yang Luo & Prof. John Todd

Contact Email Address:

yang.luo@kennedy.ox.ac.uk;

jatodd@well.ox.ac.uk

Project Overview

Our bodies rely on recognizing harmful pathogens to trigger the appropriate immune responses, a process managed by Human Leukocyte Antigens (HLA) proteins. These proteins play a crucial role in immune-related conditions, including diabetes. However, the specific roles of HLA in type 1 and type 2 diabetes—two diseases both characterised by high blood sugar but with different underlying causes—are not yet fully understood.

We have developed a state-of-the-art workflow for HLA detection and association using advanced machine learning methods. This approach allows us to accurately identify HLA variations using all forms of existing genomic data. By harnessing the power of large-scale biobanks and disease cohorts, we found this approach can greatly enhance our ability to pinpoint disease-causing variations and understand the pathophysiology of conditions such as HIV, rheumatoid arthritis and tuberculosis.

This project aims to apply these HLA methods to diabetes research. If successful, this approach could result in earlier and more precise diagnoses, reduce misclassification between type 1 and type 2 diabetes, and uncover new drug targets.

Disease Relevance

Diabetes is rising at an alarming rate globally, with over 37 million people affected as of 2022. However, studies show that more than 24% of these individuals remain undiagnosed. Additionally, data from the UK Biobank, which includes over half a million participants, indicate that more than 40% of people who develop type 1 diabetes (T1D) after age 30 are often misclassified as having type 2 diabetes (T2D). These missed and wrong diagnoses lead to inaccurate and delayed treatments.

The major histocompatibility complex (MHC) region on chromosome 6, which contains the human leukocyte antigen (HLA) and over 200 immune-related genes, plays a crucial role in differentiating T1D from T2D. Understanding the role of this region could lead to improved disease diagnosis and stratification. However, owing to its unusually high sequence variation and longer haplotypes, many studies have excluded it from their genomic analysis, leading to a missed opportunity to learn and implement MHC in clinical settings.

The incorporation of the MHC locus has already proven successful in a range of diseases such as diagnosing ankylosing spondylitis and celiac disease, as well as treating HIV and cancer. Our central hypothesis is that the inclusion of the MHC locus in the clinical management of diabetes will improve patient identification and stratification, leading to more accurate diagnoses and personalised treatment plans.

Key Technology

- For experimentalists or MHC researchers, the most natural approach to classifying individuals' HLA types is through sequencing-based typing which is timely and costly. In this project, we are advocating for a "big data" approach that leverages machine learning techniques to extract entirely new information from existing data. Given the vast amounts of genetic and healthcare data that have been collected to date and which are amenable to our techniques, our approach has the potential to transform the study of the MHC region in diabetes.
- Based in Oxford, our group has access to several large global biobanks hosted locally at Oxford (e.g., N=500K UK Biobank, N=200K China Kadoorie Biobank; N=140K Mexico City Prospective Study). This provides a unique and timely opportunity for calling and jointly analysing the MHC region across global populations to improve the current diagnosis and treatment of diabetes.
- This project has the potential to discover novel therapeutic targets for diseases by integrating human multi-omics data with laboratory data based on human tissue, cells, and preclinical models.

Training Opportunities

The successful candidate will benefit from supervision by experts in genomics, computational biology and diabetes. You will be based in the purpose-built labs at The Kennedy Institute of Rheumatology, a world-leading centre in the fields of cytokine biology and inflammation, with a strong emphasis on clinical translation.

This project is ideally suited for students with a background in statistical genetics who wish to expand their applied knowledge in the biological sciences, as well as those with a background in biology or clinical science who are interested in integrating biology with data science.

Comprehensive training will be provided in data science techniques, including statistical data analysis and visualization with R, developing computational pipelines with Python/NextFlow, and utilizing high-performance computing clusters. The student will gain expertise in analyzing advanced sequencing datasets, such as whole genome, RNA, and proteomic sequencing.

A core curriculum of lectures will provide a solid foundation in diverse subjects, including data analysis, statistical methods, and immunology summer school. In addition to institutional support, the successful applicant will benefit from the University of Oxford's college system. Students will also have the opportunity to collaborate closely with both computational and experimental scientists.

Key Publications

1) Luo, Y. et al. A high-resolution HLA reference panel capturing global population diversity enables multi-ethnic fine-mapping in HIV host response. Nature Genetics (2021)
2) Robertson, C. et al (2021) Fine-mapping, trans-ancestral, and genomic analyses identify causal variants, cells, genes, and drug targets for type 1 diabetes Nature Genet. 53, 962-971.
3) Richardson, T.G., et al (2022) Childhood body size directly increases type 1 diabetes risk based on a lifecourse Mendelian randomization approach. Nature Commun. 13, 2337.
4) Zhang JY., et al (2022) Low-dose IL-2 reduces IL-21+ T cell frequency and induces anti-inflammatory gene expression in type 1 diabetes. Nat. Commun. 13:7324. doi: 10.1038/s41467-022-34162-3.PMID: 36443294.

5) Nathan et al. Single-cell eQTL models reveal dynamic T cell state dependence of disease loci. Nature (2022)

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7.2 Integrative Multi-Omics Approach to Uncover Novel Therapeutic Targets for Heart Failure and Myocardial Infarction

Dr. Yang Luo & Dr. Jagdeep Nanchahal

Contact Email Address:

yang.luo@kennedy.ox.ac.uk;

jagdeep.nanchahal@kennedy.ox.ac.uk

Project Overview

Heart failure (HF) and myocardial infarction (MI) are major global health challenges. HF affects approximately 2.5% of adults in the United States, with an estimated 64 million people impacted worldwide. MI, often a precursor to HF, affects around 805,000 Americans annually, imposing a significant socio-economic burden. In recent decades, genomic studies have identified thousands of genetic variants that have advanced our understanding of cardiovascular disease pathogenesis. However, these genetic variants account for only a small fraction of the incidence MI and HF. Emerging evidence suggests that it is the interaction between environmental exposures and host genetics that primarily drives disease risk, severity, and therapeutic response.

This project aims to identify novel therapeutic targets for HF and MI by integrating human exposome, genome, transcriptome, and proteome data with laboratory findings from human tissues, cells, and preclinical models.

We will develop innovative machine learning models to leverage the vast amounts of multi-ancestry omics data from large-scale biobanks and consortiums. By conducting integrative analyses of these data, this approach holds the potential to enhance our understanding of the aetiology of HF and identify novel therapeutic approaches.

Disease Relevance

Myocardial infarction (MI), commonly known as a heart attack, occurs when blood flow to a part of the heart is blocked, often by a blood clot or plaque buildup, causing damage to the heart muscle. MI is a leading cause of death worldwide. In the United States, around 805,000 Americans suffer an MI annually, and of these, 200,000 are recurrent cases. MI is not just an acute event but often marks the beginning of chronic cardiovascular disease, contributing to long-term health challenges, including the development of heart failure (HF).

In low- and middle-income countries where access to timely diagnosis and treatment is limited. With more than 80% of cardiovascular-related deaths occurring in these regions, heart failure and myocardial infarction represent an urgent public health issue. Beyond mortality, the diseases impose a heavy economic burden. In the United States alone, the estimated cost of heart failure was $30.7 billion in 2012, a figure projected to reach nearly $70 billion by 2030.

Key Technology

- Recent advancements in omics data integration technologies have revolutionized biomedical research by enabling the combination of diverse datasets from genomics, transcriptomics, proteomics, metabolomics and epigenomics. These innovations allow for a

more comprehensive understanding of biological systems, disease mechanisms and personalized medicine.

- Advances in machine learning/ AI algorithms have improved the ability to handle large-scale, complex datasets, facilitating the identification of biomarkers, drug targets, and therapeutic strategies.

Training Opportunities

The successful candidate will benefit from dual supervision by an expert in genomics and computational biology, and a surgeon scientist with a focus on translational medicine. You will be based in the purpose-built labs at The Kennedy Institute of Rheumatology, a world-leading centre in the fields of cytokine biology and inflammation, with a strong emphasis on clinical translation.

This project is ideally suited for students with a background in statistical genetics who wish to expand their applied knowledge in the biological sciences, as well as those with a background in biology or clinical science who are interested in integrating biology with data science.

Comprehensive training will be provided in data science techniques, including statistical data analysis and visualization with R, developing computational pipelines with Python/NextFlow, and utilizing high-performance computing clusters. The student will gain expertise in analyzing advanced sequencing datasets, such as whole genome, RNA, and proteomic sequencing.

A core curriculum of lectures will provide a solid foundation in diverse subjects, including data analysis, statistical methods, and immunology summer school. In addition to institutional support, the successful applicant will benefit from the University of Oxford's college system. Students will also have the opportunity to collaborate closely with both computational and experimental scientists.

Key Publications

1) **Nanchahal J**, Ball C, Rombach I, Williams L, Kenealy N, Dakin H, O'Connor H, Davidson D, Werker P, Dutton SJ, Feldmann M, Lamb SE. (2022) Anti-Tumour Necrosis Factor Therapy for Early Stage Dupuytren's Disease (RIDD): a phase 2b randomised double blind, placebo-controlled trial. Lancet Rheumatology. 4(6): e407-16
2) **Luo, Y**. et al. A high-resolution HLA reference panel capturing global population diversity enables multi-ethnic fine-mapping in HIV host response. Nature Genetics (2021)
3) Ishigaki, K., Sakaue, S., Terao, C. **Luo, Y**. et al. Multi-ancestry genome-wide association analyses identify novel genetic mechanisms in rheumatoid arthritis. Nat Genet 54, 1640–1651 (2022). https://doi.org/10.1038/s41588-022-01213-w
4) Riesmeijer S, Kamali Z, Ng M, Drichel D, Piersma B, Becker K, Layton T, **Nanchahal J**, Nothnagel M, Vaez A, Hennies H, Werker P, Furniss D, Nolte I. A genome-wide association meta-analysis implicates Hedgehog and Notch signaling in Dupuytren's disease (2024). Nature Communications 15(1): 199
5) Verjee LS, Verhoekx J, Chan J, Krausgruber T, Nicolaidou V, Izadi D, Davidson D, Feldmann M, Midwood KS, **Nanchahal J** (2013)**.** Unraveling the signaling pathways promoting fibrosis in Dupuytren's disease reveals TNF as a novel therapeutic target. *Proceedings of the National Academy of Sciences, USA.* 110: E928-937

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7.3 Identifying drivers of immune-mediated disease risk and therapeutic targets via multi-omics integration

Dr. Yang Luo & Prof. Tonia Vincent

Contact Email Address:

yang.luo@kennedy.ox.ac.uk;

tonia.vincent@kennedy.ox.ac.uk

Project Overview

Proteins are the essential functional units in human metabolism, governing biological processes and forming the basis for therapies and interventions across a wide spectrum of health and disease. Recent technical advances in proteomic profiling, employing antibodies (such as Olink) or aptamer-based affinity reagents (such as SomaScan), allow high-throughput measurements of thousands of proteins. These advances enable the exploration of links between naturally occurring sequence variation in the human genome (SNPs) and protein levels (pQTL), many of which colocalize with association signals for common human diseases. By integrating existing genome-wide association studies, gene expression, and recently available proteomic data, it will become feasible to identify disease-critical variants, genes, proteins, cells, and tissues.

This project aims to investigate disease- and tissue-specific pQTLs in the context of osteoarthritis (OA). We will use newly generated proteomic data generated by the “Synovial fluid To Detect Endotypes by unbiased Proteomics in OA” consortium (STEpUP OA). This initiative is an international effort to perform proteomic analyses over 7,000 proteins in approximately 2,000 synovial fluid samples taken from 1,650 individuals with OA or at risk of OA after acute joint injury. We will integrate this with publicly available large plasma-based proteomic datasets such as the Fenland study (N = 12K) and the UK Biobank (N = 53K).

We will develop novel statistical methods to harness the wealth of genetic and proteomics data now available to help answer important questions ranging from whether OA is cartilage related to whether plasma or synovial fluid are more important for disease etiology. Through integrative analyses of genetic, expression, proteomic, and clinical data, this approach promises an improved understanding of OA pathology in a context-specific framework.

Disease Relevance

Osteoarthritis (OA) is the most common form of arthritis, affecting millions worldwide. This degenerative joint disease primarily impacts cartilage, the tissue that cushions the ends of bones within joints. According to the World Health Organization, 10%-15% of adults over 60 are affected by OA. Once regarded as an unavoidable consequence of "wear and tear," OA is now understood as a dynamic molecular response to mechanical stress, aggravated by factors such as aging, obesity, and genetics.

This project takes advantage of newly generated datasets from the STEpUP OA initiative (led by Vincent), the largest molecular and clinical dataset of its kind. Following the completion of its primary analysis, the resource enables comprehensive protein quantitative trait locus (pQTL) mapping in OA, offering valuable insights into the genetic architecture of the OA proteome. These findings could help unravel the biological mechanisms underlying proteo-

genomic discoveries and accelerate the development of OA biomarkers, predictive models, and novel therapeutics.

Key Technology

- The advent of proteomic technologies such as SomaScan and Olink has enabled assaying of large number of proteins in a high-throughput setting, allowing for the relative quantification of protein levels in population-based studies as well as in disease cohorts.
- Integrating publicaly available and disease-specific pQTL studies offers opportunity to identify disease-associated biomarkers using statistical techinques such as mendelian randomisation and colocalisation.

Training Opportunities

The successful candidate will benefit from dual supervision by an expert in genomics and computational biology, and a surgeon scientist with a focus on translational medicine. You will be based in the purpose-built labs at The Kennedy Institute of Rheumatology, a world-leading centre in the fields of cytokine biology and inflammation, with a strong emphasis on clinical translation.

This project is ideally suited for students with a background in statistical genetics who wish to expand their applied knowledge in the biological sciences, as well as those with a background in biology or clinical science who are interested in integrating biology with data science.

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Key Publications

1) Luo, Y. et al. A high-resolution HLA reference panel capturing global population diversity enables multi-ethnic fine-mapping in HIV host response. Nature Genetics (2021)
2) Zhu et al. Variants in ALDH1A2 reveal an anti-inflammatory role for retinoic acid and a new class of disease-modifying drugs in osteoarthritis. Science Translational Medicine (2022)
3) Pietzner et al. Mapping the proteo-genomic convergence of human diseases. Science (2021)
4) Sun, B.B., Chiou, J., Traylor, M. *et al.* Plasma proteomic associations with genetics and health in the UK Biobank. *Nature* **622**, 329–338 (2023). https://doi.org/10.1038/s41586-023-06592-6
5) Nathan et al. Single-cell eQTL models reveal dynamic T cell state dependence of disease loci. Nature (2022)

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7.4 Investigating the function and mechanism of adenosine methylation on non-coding RNA

Prof. Yang Shi & Prof. Richard White

Contact Email Address:

yang.shi@ludwig.ox.ac.uk;

richard.white@ludwig.ox.ac.uk

Project Overview

Gene expression is regulated at several different levels including transcription, RNA splicing and translation. In the past few decades, we have learned a great deal about how transcription is regulated by DNA-binding transcription factors and chromatin regulators, which control covalent modifications of DNA and histones. Similar to histone modifications, covalent modifications are also important for RNA regulation, but this regulation is much less well understood. One of the better studied RNA modifications is adenosine methylation at the N6 position, a modification first reported in the 1970s. Recent studies discovered that mRNA m6A methylation is mediated by the methyltransferases METTL3 and METTL14, which impacts mRNA stability, export and translation, and plays important roles ranging from stem cell biology to cancer.

Interestingly, m6A methylation is detected not only on mRNA but also on many other types of RNA, including snRNA and rRNAs. Our group is involved in the discoveries of enzymes that mediate these m6A methylation events, including PCIF1 and METTL4, which mediate mRNA and snRNA methylation, respectively. METTL4 specifically mediates m6A methylation on U2 snRNA, which is an integral component of the RNA splicing machinery, however. the function and mechanism of action of METTL4 are still poorly understood.

In this proposal, we plan to investigate the biology of m6Am on U2 snRNA, capitalizing on our discovery of the corresponding enzyme METTL4. We will take both genetic and biochemical approaches, as they are proven to be effective and complementary in addressing biological and mechanistic questions.

Disease Relevance

Potentially cancer

Key Technology

Protein purifications, RNA splicing analyses, genetic manipulation of mammalian cells including crispr. Single cell seq.

Training Opportunities

Students will learn to investigate the function and mechanism of action of a newly discovered epitranscriptomic regulator.

Key Publications

1) Chen, H., Gu, L., Orellana, EA., Wang, Y., Guo, J., Liu, Q., Wang, L., Shen, Z., Wu, H., Gregory, RI., Xing, Y., Shi, Y. METTL4 is an snRNA m^6Am methyltransferase that regulates

RNA splicing. Cell Res. 2020, 30(6):544-547. Jan 8. doi: 10.1038/s41422-019-0270-4. [Epub ahead of print].

2) Luo, Q*., Mo, JZ*., Chen, H*., Hu, ZT., Wang, BH., Wu, JB., Liang, ZY., Xie, WH., Du, KX., Peng, ML., Li, YP., Li, TY., Zhang, YY., Shi, XY., Shen, WH., Shi, Y+., Dong, AW+., Wang, HL+., Ma, JB+. Structural insights into molecular mechanism for N^6-adenosine methylation by MT-A70 family methyltransferase METTL4. Nat. Commun. 2022, Sep 26;13(1):5636. *Equal contributions; +Co-correspondence.

3) Sendinc, E and Shi, Y. (2023) RNA m6A methylation across the transcriptome. Mol Cell, 83(3):428-441.

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7.5 Developing Next-Generation Lymphoid Organoids and Lymphoid Explants for Mechanistic Studies and Translational Immunology

Dr. Pablo F. Céspedes

Contact Email Address:

pablo.cespedes@ndm.ox.ac.uk

Project Overview

Our aim is to develop the next generation of lymphoid organoids and lymphoid tissue precision-cut explants (hereafter referred to as explants) and establish them as essential toolkits for reducing and replacing animal models in immunology research. While we are focused on developing robust next-generation methods, we also aim to lead cutting-edge research and advance precision medicine. To achieve this, we will collaborate with partners to validate these systems in various immunological contexts.

Researchers within the COI umbrella primarily focus on human immunology, providing the potential candidate with numerous opportunities to collaborate and enhance the impact of our research.

Lymphoid organoids enable the reorganization of cell suspensions into follicle-like structures that support germinal center reactions, while lymphoid explants preserve tissue architecture and cell niches ex vivo. Both models have shown promise in uncovering novel immune mechanisms that regulate B cell activation and germinal center (GC) reactions. However, their broader adoption is limited, and a comprehensive body of evidence supporting their use across various immunological and non-immunological settings is lacking.

For this project, we have outlined a work plan divided into two work packages (WPs) to:

1) Benchmark and validate these methods.
2) Test their transferability as tools to elucidate fundamental mechanisms and explore clinical and molecular hypotheses.

By building a strong evidence base, we aim to further promote the adoption of these models, reducing and replacing animal experimentation and securing an immediate and long-term 3Rs impact.

WP1/Aim 1: We will validate and benchmark lymphoid organoids and explants derived from the same organ and donor, using the same stimulation regime over the same timescale. Stimulation will be based on antigens modeling viral and bacterial infections affecting the upper respiratory tract and palatine tonsils. Established primary outcome measures of adaptive immunity will include frequencies of GC-B cells, activated follicular helper T cells (TFHs), GC-TFHs, and follicular regulatory T cells (TFRs). By tracking cell subset kinetics, we will generate a comprehensive portfolio of single-cell RNA-sequencing (scRNA-seq) data, high-dimensional flow cytometry data, and high-content microscopy data.

WP2/Aim 2: We will partner with collaborators to test the transferability of these models, applying them to investigate mechanisms that maintain immune homeostasis or drive disease. Using the multimodal database generated in WP1, we will select new primary and secondary outcome measures (e.g., survival and expansion of PD-1High TFHs, plasma cell differentiation), adapt our high-dimensional flow cytometry panels, and test independent hypotheses using either one of the two 3Rs models (lymphoid organoids or lymphoid explants)

independently, or both models together in combination, depending on the specific research question. We will also incorporate the latest advancements in CRISPR/Cas9 knock-in and knockout technologies to generate lymphocytes with reporter genes, allowing us to identify and model novel mechanisms of intercellular communication.

As part of our dissemination efforts, we will create a resource article and a publicly accessible evidence base to help others choose the most suitable 3Rs model for their research. We will provide access to raw and processed data, metadata, and standardized protocols to facilitate experimental design and outcome measure selection for independent hypotheses. By building confidence in these models, we aim to encourage their widespread adoption for a variety of immunological questions and beyond, benefiting researchers globally.

Disease Relevance

- These 3Rs models (organoids and explants) will be tested in a variety of studies elucidating both fundamental mechanistic studies and their application to the understanding of disease pathogenesis and the discovery of novel therapeutics.
- The Cespedes group collaborates with many other groups under the COI umbrella, so the applications of these methods will not be limited to mechanistic studies and can be dynamically shaped to the working hypothesis of the project.
- Potential collaborations include: studying the effector mechanisms of T cell clones expressing different integrins and C-type lectins in cancer (Professor Tao Dong), inflammatory bowel disease (Dr Matthias Friedrich Lab), modelling tumor metastases, vaccinology (Dr Jack Tan, Dr Pramila Rijal) and infectious diseases (Dr Peter Wing), spatial transcriptomics for niche and pathway distribution in response to organoid and explant manipulations (Dr Matthew Bottomley), pathogenesis of malignant pleural effusions and pleural infections (Dr. Nikolaos Kanelakis), Metabolomics (Dr Adan Pinto-Fernandes).
- Potential collaborations in other areas of molecular immunology and biophysics including the study of forces regulating T-cell activation (Professor Marco Fritzsche), Protein engineering (Dr Ricardo Fernandes), and the development of new methods to study the physical communication of cells (Professor Michael L. Dustin).
- Combined with our CRISPR/Cas9, high-dimensional and high-througput microscopy approaches, the candidate will gain substantial training in cutting-edge molecular, cellular and tissue-level immunological methods. Acquiring a future-proof skillset.

Key Technology

- Compresstome for precision cut lymphoid tissue slices.
- Human lymphoid organoids.
- CRISPR/Cas9 knock-outs and knock-ins.
- Spectral flow cytometry.
- Incucyte SX5 live imaging system.
- Super-resolution microscopy.
- Super-resolution flow cytometry.
- Single-cell RNA sequencing.

- High-mass resolution DDA and DIA LC-MS/MS.
- Click chemistry.
- Metabolomics.
- Mouse experiments to model multisystemic mechanisms of immune regulation.

Training Opportunities

My goal is to be a mentor who provides opportunities to develop a future-proof skill set, grounded in rigorous scientific reasoning and the ability to channel creativity into focused efforts. I aim to strengthen your skills by fostering a dynamic learning environment that nurtures curiosity, self-awareness, and professional growth.

No prior experience is required, as candidates will be trained in cutting-edge molecular, cellular, and tissue-level immunology techniques. In addition, support will be provided for mastering informatics pipelines relevant to statistics and the analysis of complex transcriptomics and proteomics datasets.

Through collaborations, students will gain exposure to fundamental fields intersecting with immunology, such as biochemistry, biophysics, genetic engineering, protein engineering, virology, computational biology, and statistics.

Key Publications

1) Hamid, M.H.B.A., Cespedes, P.F., Jin, C. et al. Unconventional human CD61 pairing with CD103 promotes TCR signaling and antigen-specific T cell cytotoxicity. Nat Immunol 25, 834–846 (2024). https://doi.org/10.1038/s41590-024-01802-3
2) Céspedes, P.F., Jainarayanan, A., Fernández-Messina, L. et al. T-cell trans-synaptic vesicles are distinct and carry greater effector content than constitutive extracellular vesicles. Nat Commun 13, 3460 (2022). https://doi.org/10.1038/s41467-022-31160-3
3) Schneider, F., Cespedes, P.F., Karedla, N. *et al.* Quantifying biomolecular organisation in membranes with brightness-transit statistics. *Nat Commun* **15**, 7082 (2024). https://doi.org/10.1038/s41467-024-51435-1
4) David G Saliba, Pablo F Céspedes-Donoso, Štefan Bálint, Ewoud B Compeer, Kseniya Korobchevskaya, Salvatore Valvo, Viveka Mayya, Audun Kvalvaag, Yanchun Peng, Tao Dong, Maria-Laura Tognoli, Eric O'Neill, Sarah Bonham, Roman Fischer, Benedikt M Kessler, Michael L Dustin (2019) Composition and structure of synaptic ectosomes exporting antigen receptor linked to functional CD40 ligand from helper T cells eLife 8:e47528. https://doi.org/10.7554/eLife.47528

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7.6 Gene editing and base editing to cure severe forms of alpha thalassaemia

Prof. Douglas Higgs & Dr. Mira Kassouf

Contact Email Address:

doug.higgs@imm.ox.ac.uk;

mira.kassouf@imm.ox.ac.uk

Project Overview

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

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

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

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

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

Disease Relevance

As discussed above this project is relevant to common forms of inherited anaemia most importantly alpha thalassaemia which is a common form of severe anaemia throughout southeast Asia.

Key Technology

- Mouse Genetics
- Cell culture of ES Cells, Erythroid Cells and Immortalised Cell lines
- Flow Sorting
- All techniques of routine molecular biology and genome engineering
- Analysis of transcriptomics and Epigenetics
- Analysis of 3D genome structure
- Crispr-mediated gene HDR
- Crispr-mediated base editing
- Single cell biology
- Microscopy including single moleculae and super-resolution microscopy
- Computational analysis

Training Opportunities

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

Key Publications

1) Amid A, Liu S, Babbs C, Higgs DR. Hemoglobin Bart's hydrops fetalis: charting the past and envisioning the future. Blood. 2024
2) Blayney JW, Francis H, Rampasekova A, Camellato B, Mitchell L, Stolper R, Cornell L, Babbs C, Boeke JD, Higgs DR, Kassouf M. Super-enhancers include classical enhancers and facilitators to fully activate gene expression. Cell. 2023 Dec
3) Badat M, Ejaz A, Hua P, Rice S, Zhang W, Hentges LD, Fisher CA, Denny N, Schwessinger R, Yasara N, Roy NBA, Issa F, Roy A, Telfer P, Hughes J, Mettananda S, Higgs DR, Davies JOJ. Direct correction of haemoglobin E β-thalassaemia using base editors. Nature Communications.
4) King AJ, Songdej D, Downes DJ, Beagrie RA, Liu S, Buckley M, Hua P, Suciu MC, Marieke Oudelaar A, Hanssen LLP, Jeziorska D, Roberts N, Carpenter SJ, Francis H, Telenius J, Olijnik AA, Sharpe JA, Sloane-Stanley J, Eglinton J, Kassouf MT, Orkin SH, Pennacchio LA,

Davies JOJ, Hughes JR, Higgs DR, Babbs C. Reactivation of a developmentally silenced embryonic globin gene. Nature Communications. 2021

5) Hua P, Badat M, Hanssen LLP, Hentges LD, Crump N, Downes DJ, Jeziorska DM, Oudelaar AM, Schwessinger R, Taylor S, Milne TA, Hughes JR, Higgs DR, Davies JOJ. Defining genome architecture at base-pair resolution. Nature. 2021

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7.7 Assessing the long-term health, social and educational costs of prematurity: evidence from representative English cohorts

Prof. Ramon Luengo-Fernandez & Prof. Oliver Rivero-Arias

Contact Email Address:

ramon.luengo-fernandez@dph.ox.ac.uk;

oliver.rivero@npeu.ox.ac.uk

Project Overview

With improvements in maternal and neonatal healthcare, the number of babies born prematurely (birth at less than 37 weeks gestation) is increasing. In the UK, the proportion of babies born prematurely is 7.6%, higher than in other Western European countries. Prematurity poses a serious risk to a baby's health as many organs will not have fully developed, with consequences potentially stretching over their lifetimes. A negative association has been found to exist between gestational age at birth and the risk of subsequent health and developmental problems, such cerebral palsy and hearing/vision problems. Therefore, the costs to the health and social care systems, as well as to the education sector are likely to be considerable. Although there is plenty of evidence showing the adverse health and education challenges of prematurity, there is limited evidence on the overall costs to these sectors, and that of particular conditions associated with prematurity, such as cerebral palsy or hypoxic ischemic encelopathy.

This project would suit a candidate with a strong interest in working with large, centrally-linked, representative datasets, who is dynamic and can work independently to progress the project between supervision meetings.

Disease Relevance

It is estimated that nearly 58,000 babies are born prematurely (i.e. before 37 weeks gestation) in the UK every year. This represents 1 in every 13 babies. In addition, ethnic inequalities exists, with babies from black backgrounds being most at risk of prematurity. Many of these babies will require care more advanced levels of care, such as neonatal units and increased specialist care. However, the impact of prematurity is not only confined to the first weeks of a baby's life, with premature babies at increased long-term risk of conditions such as behavioural difficulties, health problems or cerebral palsy. Research has also found that the earlier a baby is born the higher their risk of having special educational needs at school.

Key Technology

This doctoral programme will provide the student access to large, patient-level, linked datasets. As part of the programme, the student will be able to use ECHILD, a recently set linked dataset bringing together not only hospital care records, but education and social care information, so that the costs of prematurity can be assessed using a wide perspective. The student will also have access to the Clinical Practice Research Datalink (CPRD), which captures contacts with the National Health Service (NHS), including primary and community care.

Training Opportunities

This doctoral programme will provide experience and training in literature review methods, data management and statistical methods to handle and synthesise large datasets. The student will also apply econometric methods to handle resource use and cost information, using panel data and statistical methods to evaluate differences across regions in England, and across conditions associated with prematurity. The student will conduct a programme of work that will involve:

1) A comprehensive review of the literature to identify previous studies assessing the costs of prematurity in the UK and elsewhere.
2) The use of routine datasets to quantify the likely health, social and education costs of prematurity in England.
3) The use of appropriate econometric and statistical techniques to understand within-regional and within-condition variations associated with the cost of prematurity in England.

Key Publications

1) Petrou S, Johnson S, Wolke D, Marlow N. The association between neurodevelopmental disability and economic outcomes during mid-childhood. Child Care Health Dev 2013;39:345-57.
2) Rivero-Arias O , Eddama O, Azzopardi D, Edwards AD, Strohm B, et al. Hypothermia for perinatal asphyxia: trial-based resource use and costs at 6-7 years. Arch Dis Child Fetal Neonatal Ed 2019; 104:F285-F292.
3) Petrou, S., Yiu, H.H., and Kwon, J., Economic consequences of preterm birth: a systematic review of the recent literature (2009-2017). Arch Dis Child, 2019. 104(5): p. 456-465.
4) Mangham LJ, Petrou S, Doyle LW, Draper ES, Marlow N. The cost of preterm birth throughout childhood in England and Wales. Pediatrics 2009;123:e312-e327
5) Mc Grath-LoneL, Libuy N, Harron K, et al. Data resource profile: The Education and Child Health Insights from Linked Data (ECHILD) Database. International Journal of Epidemiology 2022;51:17-17f genome architecture at base-pair resolution. Nature. 2021

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7.8 Investigating UCHL1's Role in Innate Immune Signalling

Prof. Benedikt Kessler & Dr. Andreas Damianou

Contact Email Address:

benedikt.kessler@ndm.ox.ac.uk;

andreas.damianou@ndm.ox.ac.uk

Project Overview

In our laboratory, we recently identified an interaction between Ubiquitin C-terminal Hydrolase L1 (UCHL1) and NLRP3, indicating a possible regulatory role of UCHL1 in the NLRP3 inflammasome activation process and interleukin 1 beta (IL-1□) production. UCHL1, a C12 deubiquitinating enzyme (DUB) family member, is primarily expressed in neuronal cells including microglia, the 'macrophages' of the brain. We confirmed UCHL1 expression in iPNC-derived microglia and macrophages using single-cell proteomics, suggesting a broader role in immune cells. Despite this, the role of UCHL1 in innate immunity remains unclear. UCHL1 mutations have been linked to neurodegenerative diseases, such as Parkinson's and Alzheimer's, but the precise mechanisms are still unknown.

In this proposal, we aim to investigate further the role of UCHL1 in innate immunity, in particular NLRP3 inflammasome activation and IL-1□ production, by generating THP1-derived macrophage cell lines with UCHL1 knockout (KO), and overexpressing either wild-type (WT) UCHL1, catalytically inactive UCHL1, or several other UCHL1 mutants associated with neurodegenerative diseases.

Objectives:

Proteomic and Ubiquitome Analysis:

We plan to perform proteomic analysis and diGly enrichment to monitor both the proteome and ubiquitome in naïve and activated THP1 macrophages. These analyses will help us understand the impact of UCHL1 on protein stability and turnover, both of which are critical in immune responses.

Functional Characterization of UCHL1 Mutants:

By generating THP1 cells expressing various UCHL1 mutants, we aim to study how specific mutations affect UCHL1's role in regulating the NLRP3 inflammasome. This will show how UCHL1 dysfunction influences immune cell activity and its broader role in inflammation, potentially contributing to neurodegeneration.

This research will provide crucial insights into UCHL1's function in innate immunity, helping us better understand its connection to immune regulation and neurodegenerative diseases. Ultimately, this work could reveal new therapeutic targets for Parkinson's, Alzheimer's, and inflammatory diseases.

Disease Relevance

This research will provide crucial insights into UCHL1's function in innate immunity, helping us better understand its connection to immune regulation and neurodegenerative diseases. Ultimately, this work could reveal new therapeutic targets for Parkinson's, Alzheimer's, and inflammatory diseases.

Key Technology

- Ubiquitomics: We will leverage ubiquitin-specific proteomics to explore the role of UCHL1 in regulating the ubiquitin landscape.
- Advanced Mass Spectrometry for Proteomics: High-resolution mass spectrometry will perform in-depth proteomic analysis, allowing us to identify and quantify protein interactions and post-translational modifications. Single-cell proteomics may also be used.
- CRISPR/Cas9 DUB Library: We will employ a CRISPR/Cas9-mediated knockout library targeting deubiquitinating enzymes (DUBs), including UCHL1, to systematically investigate their roles in innate immunity.
- Proximity Proteomics: APEX2 proximity labeling techniques will be used to map the interaction network of UCHL1 in living cells, providing a spatial context for its involvement in immune pathways.

Training Opportunities

- This project will provide valuable training in essential lab techniques such as tissue culture, protein expression, gene editing, and proximity labelling. Students will also learn PTM enrichment methods for mass spectrometry analysis in proteomics.
- Additionally, participants will gain hands-on experience with molecular biology tools, including Western blotting, immunoprecipitation, and creating genetically modified cell lines. There will also be opportunities to explore single-cell proteomics and work with iPSC-derived cell lines, offering a broad range of technical and analytical skills.
- Students will also have the opportunity to learn how to analyse complex datasets from proteomics and ubiquitomics studies using R, equipping them with essential bioinformatics skills.

Key Publications

1) Liang, Z., Damianou, A., Vendrell, I., Jenkins, E., Lassen, F.H., Washer, S.J., Grigoriou, A., et al., 2024. Proximity proteomics reveals UCH-L1 as an essential regulator of NLRP3-mediated IL-1β production in human macrophages and microglia. Cell Reports, 43(5), p.114152. doi: 10.1016/j.celrep.2024.114152.
2) Liang, Z., Damianou, A., Di Daniel, E. and Kessler, B.M., 2021. Inflammasome activation controlled by the interplay between post-translational modifications: emerging drug target opportunities. Cell Communication and Signaling, 19(1), p.23. doi: 10.1186/s12964-021-00696-0.
3) Dietz, L., Ellison, C.J., Riechmann, C., Cassidy, C.K., Felfoldi, F.D., Pinto-Fernández, A., et al., 2023. Structural basis for SMAC-mediated antagonism of caspase inhibition by the giant ubiquitin ligase BIRC6. Science, 379(6637), pp.1112-1117. doi: 10.1126/science.ade8840.
4) Pinto-Fernandez, A., Salio, M., Partridge, T., Chen, J., Vere, G., Greenwood, H., et al., 2021. Deletion of the deISGylating enzyme USP18 enhances tumour cell antigenicity and radiosensitivity. British Journal of Cancer, 124(4), pp.817-830. doi: 10.1038/s41416-020-01167-y.
5) Turnbull, A.P., Ioannidis, S., Krajewski, W.W., Pinto-Fernandez, A., Heride, C., Martin, A.C.L., et al., 2017. Molecular basis of USP7 inhibition by selective small-molecule inhibitors. Nature, 550(7677), pp.481-486. doi: 10.1038/nature24451.

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Nuffield Department of Medicine,
University of Oxford,
Old Road Campus, Oxford,
OX3 7BN, United Kingdom
Tel: +44 (0)1865 287773
www.camsoxford.ox.ac.uk