CHINESE ACADEMY of MEDICAL SCIENCES OXFORD INSTITUTE

NUFFIELD DEPARTMENT of MEDICINE

UNIVERSITY OF OXFORD

CSC-COI Scholarship DPhil Project Proposals

Enrolment Year: 2024

28 Projects Available

- ☐ Cancer: 10 projects
- ☐ Viral Infection: 9 projects
- ☐ Other Themes: 9 projects

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1. Cancer Theme

1.1 Targeting Deubiquitylating Enzymes for Enhanced Cancer Immunotherapies

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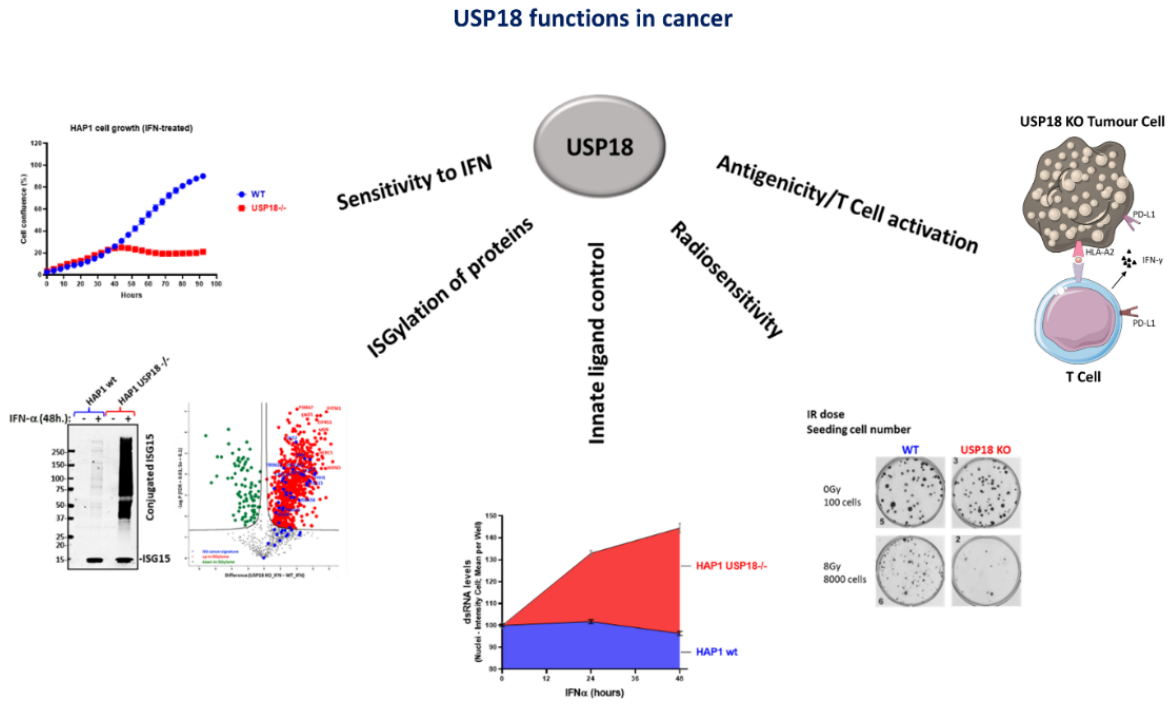
Project Overview

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

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



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

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

Disease Relevance

Cancer Immunotherapy and Viral infections.

Key Technology

Proteomics, Ubiquitomics, Lipidomics, and Immunology.

Training Opportunities

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

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

Key Publications

- Pinto-Fernandez A, Salio M, Partridge T, Chen J, Vere G, Greenwood H, Olie CS, Damianou A, Scott HC, Pegg HJ, Chiarenza A, Díaz-Saez L, Smith P, Gonzalez-Lopez C, Patel B, Anderton E, Jones N, Hammonds TR, Huber K, Muschel R, Borrow P, Cerundolo V, Kessler BM. Deletion of the deISGylating enzyme USP18 enhances

tumour cell antigenicity and radiosensitivity. Br J Cancer. 2021 Feb;124(4):817-830. doi: 10.1038/s41416-020-01167-y. Epub 2020 Nov 20. PMID: 33214684; PMCID: PMC7884788.

- Turnbull AP, Ioannidis S, Krajewski WW, Pinto-Fernandez A, Heride C, Martin ACL, Tonkin LM, Townsend EC, Buker SM, Lancia DR, Caravella JA, Toms AV, Charlton TM, Lahdenranta J, Wilker E, Follows BC, Evans NJ, Stead L, Alli C, Zarayskiy VV, Talbot AC, Buckmelter AJ, Wang M, McKinnon CL, Saab F, McGouran JF, Century H, Gersch M, Pittman MS, Marshall CG, Raynham TM, Simcox M, Stewart LMD, McLoughlin SB, Escobedo JA, Bair KW, Dinsmore CJ, Hammonds TR, Kim S, Urbé S, Clague MJ, Kessler BM, Komander D. Molecular basis of USP7 inhibition by selective small-molecule inhibitors. Nature. 2017 Oct 26;550(7677):481-486. doi: 10.1038/nature24451. Epub 2017 Oct 18. PMID: 29045389; PMCID: PMC6029662.
- Pinto-Fernandez A, Kessler BM. DUBbing Cancer: Deubiquitylating Enzymes Involved in Epigenetics, DNA Damage and the Cell Cycle As Therapeutic Targets. Front Genet. 2016 Jul 28;7:133. doi: 10.3389/fgene.2016.00133. PMID: 27516771; PMCID: PMC4963401.
- Vere G, Kealy R, Kessler BM, Pinto-Fernandez A. Ubiquitomics: An Overview and Future. Biomolecules. 2020 Oct 17;10(10):1453. doi: 10.3390/biom10101453. PMID: 33080838; PMCID: PMC7603029.
- Jones HBL, Heilig R, Davis S, Fischer R, Kessler BM, Pinto-Fernández A. ABPP-HT*-Deep Meets Fast for Activity-Based Profiling of Deubiquitylating Enzymes Using Advanced DIA Mass Spectrometry Methods. Int J Mol Sci. 2022 Mar 17;23(6):3263. doi: 10.3390/ijms23063263. PMID: 35328685; PMCID: PMC8955990. https://doi.org/10.3390/biom10101453.

1.2 Therapeutic Target and Biomarker Discovery for Malignant Pleural Mesothelioma and Malignant Pleural Effusions in Patient-derived Specimens using Cutting-edge Proteomics and Ubiquitomics

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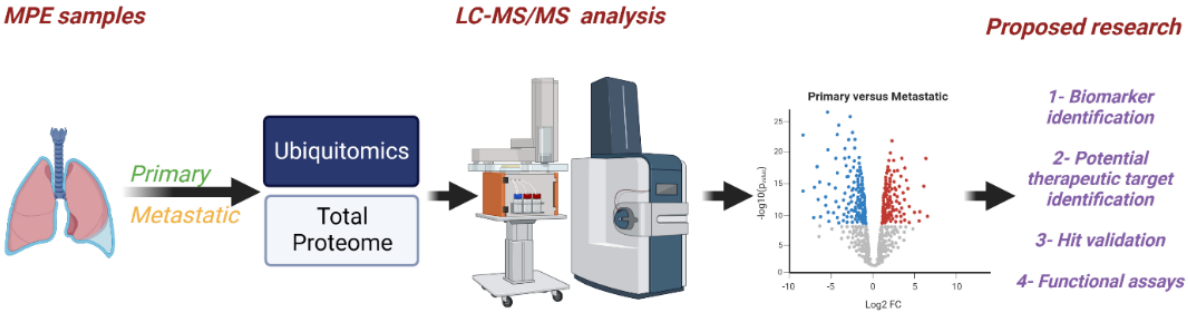
Project Overview

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

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

We will use advanced mass-spectrometry proteomics and ubiquitomics to elucidate the molecular landscape of MPE patient-derived specimens, for both cells and fluids (pleural effusion and paired blood samples), with the aim to identify therapeutic targets and disease biomarkers. The analysis of the proteome will allow us to identify differentially expressed proteins and pathways in the malignant samples, and thanks to the ubiquitomic analysis, we will focus on cancer-specific changes inside the ubiquitin system. Our group is interested in translational studies targeting deubiquitylating enzymes (DUBs), the proteases that revert

ubiquitylation. For instance, and as a proof-of-concept, we have already collected and analysed the proteome of cancer cells present in the MPE of cancer patients. The results from this study can potentially identify ground-breaking disease biomarkers, including prognostic factors, and therapeutic targets.



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

Disease Relevance

The results from this study can potentially identify ground-breaking disease biomarkers, including prognostic factors, and therapeutic targets in the context of Malignant Pleural Mesothelioma and Malignant Pleural Effusions.

Key Technology

Proteomics, Ubiquitomics, and Bionformatics.

Training Opportunities

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

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

Key Publications

- Bibby AC, Dorn P, Psallidas I, Porcel JM, Janssen J, Froudarakis M, Subotic D, Astoul P, Licht P, Schmid R, Scherpereel A, Rahman NM, Cardillo G, Maskell NA. ERS/EACTS statement on the management of malignant pleural effusions. Eur Respir J. 2018 Jul 27;52(1):1800349. doi: 10.1183/13993003.00349-2018. PMID: 30054348.
- Psallidas I, Kanellakis NI, Gerry S, Thézénas ML, Charles PD, Samsonova A, Schiller HB, Fischer R, Asciak R, Hallifax RJ, Mercer R, Dobson M, Dong T, Pavord ID, Collins GS, Kessler BM, Pass HI, Maskell N, Stathopoulos GT, Rahman NM. Development and validation of response markers to predict survival and pleurodesis success in patients with malignant pleural effusion (PROMISE): a multicohort analysis. Lancet Oncol. 2018 Jul;19(7):930-939. doi: 10.1016/S1470-2045(18)30294-8. Epub 2018 Jun 13. PMID: 29908990.
- Kanellakis NI, Asciak R, Hamid MA, Yao X, McCole M, McGowan S, Seraia E, Hatch S, Hallifax RJ, Mercer RM, Bedawi EO, Jones S, Verrill C, Dobson M, George V, Stathopoulos GT, Peng Y, Ebner D, Dong T, Rahman NM, Psallidas I. Patient-derived malignant pleural mesothelioma cell cultures: a tool to advance biomarker-driven treatments. Thorax. 2020 Nov;75(11):1004-1008. doi: 10.1136/thoraxjnl-2020-215027. Epub 2020 Sep 17. PMID: 32943495; PMCID: PMC7569377.
- Pinto-Fernandez A, Salio M, Partridge T, Chen J, Vere G, Greenwood H, Olie CS, Damianou A, Scott HC, Pegg HJ, Chiarenza A, Díaz-Saez L, Smith P, Gonzalez-Lopez C, Patel B, Anderton E, Jones N, Hammonds TR, Huber K, Muschel R, Borrow P, Cerundolo V, Kessler BM. Deletion of the deISGylating enzyme USP18 enhances tumour cell antigenicity and radiosensitivity. Br J Cancer. 2021 Feb;124(4):817-830. doi: 10.1038/s41416-020-01167-y. Epub 2020 Nov 20. PMID: 33214684; PMCID: PMC7884788.
- Vere G, Kealy R, Kessler BM, Pinto-Fernandez A. Ubiquitomics: An Overview and Future. Biomolecules. 2020 Oct 17;10(10):1453. doi: 10.3390/biom10101453. PMID: 33080838; PMCID: PMC7603029.

1.3 Evaluating cellular mechanisms and translational prospect of transient co-stimulatory signals of antigen-specific T cell responses

Prof. Tao Dong & Dr. Megat Hamid

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Project Overview

Tissue-resident memory (Trm) T cells, especially CD103+ Trm TILs has been well-established to associate with better clinical survival, especially in early-stage lung cancer patients. However, it remains unclear the immunophenotype, cellular and molecular mechanisms that is driving the T cell immune outcome and in providing protective immunity. This project will focus on evaluating the role of Trm biomarkers in contributing towards anti-tumor immune activities.

We have recently discovered a novel integrin subunit, CD61, which is transiently expressed on antigen-specific cytotoxic CD8+ T cells present at the T cell synaptic interface, leading to enhanced T cell killing and mitigation of tumor growth. Still, we have limited insight into the essential regulation of this protein on human CD8+ T cells. In particular, determining the specific ligand of this integrin subunit of T cells would be important to relate to clinical immunophenotype of cancer and immune cells. This will be done using in vitro cultured antigen-specific T cell clones and TCR-T cells already generated in the lab, as well as using CRISPR method to evaluate the potential ligand such as E-Cadherin on targeted antigenic cancer cells available in the lab (in collaboration with Dr Pablo Cespedes group at COI). Collaboration with Dr Ricardo Fernandes group at COI to generate biotinylated tetramer of suspected ligand protein and staining of antigen-specific T cells on the potential ligation will also be undertaken.

Additionally, the contribution of this novel integrin on cytotoxic T cells in elevating anti-tumor immunity suggest that it could be a potential target for immunotherapy. Therefore, this project will evaluate the translational prospect of targeting CD61, using two approaches of bi-specific diabody and engineering of CD61-expressing (either constitutively or transiently) on NY-ESO-1-specific TCR-T cells. Confirmation of functions initially at in vitro level will be supplemented and confirmed using in vivo model and ex vivo treatment of cells isolated from lung cancer patients.

Disease Relevance

Lung cancer remains one of the leading causes of mortality worldwide and despite current immunotreatment shown recovery of CD103+ Trm TILs, not all patients exhibited improved clinical outcome. Therefore, evaluating the T cell immune phenotype and diversity of the TILs are important towards determining the specific biomarkers of cytotoxic T cells that are responsible for promoting protective immunity.

Key Technology

Multicolour flow cytometry, T cell functional assays (in vitro, ex vivo), in vivo xenograft model, CRISPR-Cas9, molecular techniques, fresh paired samples of lung cancer, Engineering of T cells.

Training Opportunities

Student will be trained in culturing antigen-specific T cell clones and TCR-T, functional assays in vitro, molecular techniques in preparing the plasmids for transduction/engineering of T cells, processing of fresh paired samples of lung cancer patients.

Key Publications

- Self-maintaining CD103+ cancer-specific T cell are highly energetic with rapid cytotoxic and effector responses, Cancer Immunol Res, 2020.
- Enriched HLA-E and CD94/NKG2a interaction limit antitumor CD8+ tumor-infiltrating T lymphocyte responses, Cancer Immunol Res, 2019
- Defective Interfereon gamma production by tumor-specific CD8+ T cells is associated with 5’methylcytosine-guanine hypermethylatin of interferon gamma promoter, Frontiers in Immunology, 2020
- Human cancer germline antigen-specific cytotoxic T cell – what we can learn from patinet, Cel & Mol Immnul, 2020
- An unconventional CD61 integrin expression on human antigen-specific CD8+ T cells promotes anti-tumor effector and cytotoxic immunity, in revision

1.4 Investigating the link between CD8+ T cell avidity, TIL function and anti-cancer responses in oesophageal cancer

Prof. Tim Elliott, Prof. Andrew McMichael, Dr. Hongbing Yang & Dr. Richard Owen

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Project Overview

We have recently discovered that tumour antigen-specific tumour infiltrating lymphocytes (TIL) with low avidity are preferentially expanded in response to both Treg depletion and anti-PD1 immunotherapy and are curative (1,2). These CTL have a phenotype similar to stem-like resident memory T cells (TRM) which have been identified in a variety of different cancers including lung cancer where they have been associated with improved survival (3,4). They have also been observed within a limited cohort of oesophageal adenocarcinomas or OACs (5).

We have demonstrated that OACs are often highly infiltrated by a population of TRM-like PD-1+ CD39+CD103+TIM3-LAG3- antigen experienced CD8+ T lymphocytes, and that possession of a lymphocyte population enriched for this phenotype is associated with improved survival after surgery.

This project therefore aims to:

a) determine whether these T cells also have a low avidity like their counterparts in the mouse model
b) might be specifically induced by rational vaccination strategies.Disease Relevance

Disease Relevance

Oesophageal cancer is a major disease burden globally, with 473 000 new cases of oesophageal cancer and 436 000 deaths due to oesophageal cancer in 2017. Only 10% 10 year survival rate.

Key Technology

- T cell isolation and culture
- Multiparameter flow cytometric sorting
- Spatial transcrptomics and proteomics
- Epitope discovery
- Z-Movi cell avidity ultrasonics

Training Opportunities

Training in a wide range of cellular and molecular techniques using human patient samples. Quantitative biology including fundamental bioinformatic and data analytics. Multidisciplinary team working across the spectrum from molecular mechanism, computational modelling, preclinical animal models to human patient samples.

Key Publications

- Sugiyarto G, Prossor D, Dadas O, Arcia-Anaya ED, Elliott T, James E. Protective low-avidity anti-tumour CD8+ T cells are selectively attenuated by regulatory T cells. Immunother Adv. 2020 Nov 25;1(1):ltaa001. doi: 10.1093/immadv/ltaa001. PMID: 33748824; PMCID: PMC7958313.
- Sugiyarto G, Lau D, Hill SL, Arcia-Anaya D, Boulanger DSM, Parkes E, James E, Elliott T. Reactivation of low avidity tumor-specific $CD8^{+}$ T cells associates with immunotherapeutic efficacy of anti-PD-1. J Immunother Cancer. 2023 Aug;11(8):e007114. doi: 10.1136/jitc-2023-007114.
- Clarke J, Panwar B, Madrigal A, Singh D, Gujar R, Wood O, et al. Single-cell transcriptomic analysis of tissue-resident memory T cells in human lung cancer. J Exp Med. 2019;216(9):2128-49.
- Thommen DS, Koelzer VH, Herzig P, Roller A, Trefny M, Dimeloe S, et al. A transcriptionally and functionally distinct PD-1(+) CD8(+) T cell pool with predictive potential in non-small-cell lung cancer treated with PD-1 blockade. Nat Med. 2018;24(7):994-1004.
- Croft W, Evans RPT, Pearce H, Elshafie M, Griffiths EA, Moss P. The single cell transcriptional landscape of esophageal adenocarcinoma and its modulation by neoadjuvant chemotherapy. Mol Cancer. 2022;21(1):200.

1.5 A Multidimensional Evaluation of Regulatory T Cells in Melanoma-Induced Immune Evasion

Prof. Fadi Issa, Prof. Joanna Hester & Dr. George Adigbli

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Project Overview

Abstract

Melanoma is the most lethal skin cancer and has the fastest rising incidence of all cancers in the UK. Its aggressive nature stems in part from its propensity to form thick tumours that are adept at inducing immune escape. T cell receptor (TCR) research has provided critical insights into anti-tumour immunity in melanoma, enabling approaches like TCR-engineered T cells or sequencing-based response monitoring. However, little is known about melanoma reactive TCRs on regulatory T cells (Tregs). These are dominant immunosuppressive cells in melanoma, yet their antigen specificities and clonality remain largely unexplored. This project aims to combine TCR and Treg analysis to provide new perspectives on Treg-mediated immunosuppression in melanoma.

Background

Melanoma is the most lethal form of skin cancer, and its incidence is rising faster than any other cancer in the UK. Early stage I tumours that are confined to the skin can rapidly progress to thicker stage II tumours before metastasising to nearby lymph nodes (stage III) or distant organs (stage IV). Despite being localised to the skin, stage II melanoma marks a critical transition point toward more aggressive disease. Patients with thinner stage II melanoma are often cured by removal of the tumour, while those with thicker stage IIB/C tumours have worse outcomes than some patients with thinner stage IIIA disease—the low-risk paradox[1].

The immune system plays a key role in controlling melanoma progression. Tumour-infiltrating lymphocytes (TILs) are immune cells that can recognise and eliminate cancer cells[2]. We recently identified linear associations between tumour thickness and TILs. As thickness increases, non-brisk TILs (low density and sparsely distributed) increase while brisk TILs (high density and well dispersed) decrease. Among stage II patients, this translated into a pattern where melanomas with non-brisk TILs had the highest progression rates, remarkably, even higher than tumours lacking TILs altogether. We hypothesise that non-brisk TILs in stage II melanoma may reflect developing immune evasion induced by the thicker tumours.

This is often mediated by regulatory T cells (Tregs), which suppress anti-tumour immunity by inhibiting conventional T cells[3,4]. This can take place in the tumour microenvironment or in the lymph nodes, through impairment of T cell activation.

The potential DPhil candidate will investigate Treg development in melanoma through sequencing the T cell receptors (TCR) of Tregs and conventional T cells in the blood and lymphatic fluid, and in primary tumour and lymph nodes, using advanced spatial profiling techniques. This multi-dimensional research will yield valuable insights into Treg biology and bring potential to unveil novel prognostic biomarkers and identify therapeutic avenues targeting Tregs to augment anti-tumour immunity.

Academic Value of the Research and Funding Justification

Funding for a DPhil student will enable us to transform understanding of melanoma immunology, by providing insights into the mechanisms of Treg-mediated immune evasion, the potential neoantigens driving their enhanced function, and their impact on metastatic potential. Progress on this challenging problem requires a multidisciplinary team with expertise across translational immunology, surgical oncology, experimental biology, image analysis and bioinformatics. The funds will cover the costs of a graduate student who will carry out collaborative research between the COI and the NDS.

Collaborations

The project will initiate a new collaboration between Professors Dong (COI), Issa (COI/NDS) and Hester (NDS) and Mr Oliver Cassell (Oxford University Hospitals (OUH) Plastic Surgery Dept). The DPhil candidate will also benefit from mentorship from a post-doctoral academic clinician co-supervisor—Dr George Adigbli, academic plastic surgery registrar at OUH.

The candidate will make frequent visits to the Dong/Issa/Hester labs at the COI and IDRM where they will interact with lab members investigating Treg immunology and melanoma. This will enable the candidate to learn the relevant melanoma biology, experimental techniques and data analysis methods, and to contribute to the experimental design. The team will meet with the candidate on a weekly basis.

Translational potential

This project will provide new insights into the processes driving melanoma metastasis and immunotherapy resistance, through an in-depth exploration of Treg-mediated immune evasion and potential neoantigens. It will generate new methodologies for analysing TILs and strengthen expertise in multidisciplinary approaches to tackling cancer.

References

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- Lee, S.J.*, et al.* The clinical significance of tumor-infiltrating lymphocytes and microscopic satellites in acral melanoma in a korean population. *Ann Dermatol* 25, 61-66 (2013).
- Jacobs, J.F., Nierkens, S., Figdor, C.G., de Vries, I.J. & Adema, G.J. Regulatory T cells in melanoma: the final hurdle towards effective immunotherapy? *The lancet oncology* 13, e32-42 (2012).
- Xydia, M.*, et al.* Common clonal origin of conventional T cells and induced regulatory T cells in breast cancer patients. *Nature Communications* 12, 1119 (2021).

Disease Relevance

Melanoma is an aggressive skin cancer with rising global incidence rates, which presents a substantial public health challenge[1]. As melanomas progress, they acquire the ability to evade immune-mediated elimination. Tregs have emerged as critical mediators of immune suppression in advanced melanoma, but their precise roles remain poorly defined. By providing high-resolution insights into the origins, evolution and antigen specificities of Tregs during melanoma progression, this project can uncover new prognostic biomarkers and opportunities to therapeutically target Tregs to reinvigorate anti-tumour immunity.

The focus on Tregs in melanoma makes this research particularly relevant, as melanoma has been at the vanguard of cancer immunotherapy development. Findings from this study will provide foundational knowledge to guide next-generation immunotherapies designed to counteract Treg-mediated immune evasion. More broadly, the insights gained could inform immunotherapy strategies across multiple solid tumour types where Tregs represent formidable barriers to efficacy. This timely project, which leverages cutting-edge technologies to address a critical gap in understanding immune suppression in melanoma, brings great potential to improve patient outcomes.

Key Technology

RNA sequencing - Profiling gene expression in bulk and individual cells isolated from blood, lymphatic fluid, tumour and lymph node samples to identify distinct immune cell subsets and signalling pathways.

T cell receptor (TCR) sequencing - Characterising the diversity and clonality of T cells responding to melanoma antigens by sequencing the TCR locus.

Multi-omic spatial profiling - Integrating spatial information with gene expression profiles from tissue sections, enabling spatially resolved tumour-immune profiling (10X & NanoString).

Bioinformatics and machine learning - For analysing and interpreting high-dimensional data sets generated by the techniques above and identifying associations and patterns related to clinical outcomes.

Training Opportunities

This exciting project offers rich training opportunities for the DPhil candidate, supervisory team and collaborating departments.

Fundamental research skills the candidate will receive training in include experimental design, in vitro cell-based assays, data analysis, scientific writing and communication, and multidisciplinary research. In addition, they will be trained in advanced techniques such as RNA sequencing, spatial biology, multiparameter image analysis and bioinformatics.

Within the Nuffield Department of Surgical Sciences, the candidate will be part of the Institute for Developmental and Regenerative Medicine, where they will take part in weekly translational immunology lab meetings (TRIG lab) and research skills training sessions and formal weekly seminars and journal clubs. They will also enjoy access to oncology meetings and relevant seminars arranged by Oxford Cancer, and to clinically oriented student-run activities in the Oxford Plastic Surgery Society and the Oxford Surgical Society.

For the post-doctoral co-supervisor, this will be a valuable opportunity to gain mentoring experience and develop leadership skills.

The departments will benefit from this new collaboration, bringing opportunities to share expertise in translational immunology and melanoma research.

Key Publications

- Letter to the Editor: Complex Lymphatic Drainage in Head and Neck Cutaneous Melanoma and SLNB Outcomes. G. Adigbli, L. Woolley and F. Issa. JAMA Otolaryngol Head Neck Surg 2023 Vol. 149 Issue 9 Pages 853-854.
- The changing landscape in management of desmoplastic melanoma. J. A. Dunne and G. Adigbli. J Surg Oncol 2021.

- Spatial transcriptomic characterization of COVID-19 pneumonitis identifies immune circuits related to tissue injury. A. R. Cross, C. E. de Andrea, M. Villalba-Esparza, M. F. Landecho, L. Cerundolo, P. Weeratunga, et al. JCI Insight 2023 Vol. 8 Issue 2.
- Development of LT-HSC-Reconstituted Non-Irradiated NBSGW Mice for the Study of Human Hematopoiesis In Vivo. G. Adigbli, P. Hua, M. Uchiyama, I. Roberts, J. Hester, S. M. Watt, et al. Frontiers in Immunology 2021 Vol. 12 Issue 836
- Chimeric antigen receptor-modified human regulatory T cells that constitutively express IL-10 maintain their phenotype and are potently suppressive. Y. R. Mohseni, A. Saleem, S. L. Tung, C. Dudreuilh, C. Lang, Q. Peng, et al. European Journal of Immunology 2021 Vol. 51 Issue 10 Pages 2522-2530

1.6 Decoding the epi-transcriptome in cancer

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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. Interest in understanding the functions of RNA modifications, as well as the related molecular mechanisms, has been growing, driving progress in developing chemical and biochemical tools to detect specific modifications within the transcriptomes. New technologies are important for uncovering biological functions, and they can also drive conceptual revolutions. From the perspective of cancer, epigenetic regulation plays pivotal roles in tumour development and maintenance, and aberrant patterns of epigenetic modifications are hallmarks of cancer. Therefore, mapping epigenetic modifications becomes crucial not only to understand the molecular basis of cancer, but also to develop diagnostic and therapeutic opportunities.

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 the revolutionary TAPS technology for bisulfite-free and base-resolution direct sequencing of DNA methylation. Recently, we developed CAPS+ for improved DNA hydroxymethylation sequencing. Furthermore, we applied our tools to cell-free DNA for non-invasive early cancer detection.

Our future research aims to develop novel technologies to sequence important RNA epitranscriptomic modifications with biological significance, such as N6-methyladenosine (m6A), pseudouridine (ψ), 5-methylcytidine (m5C), and N7-methylguanosine (m7G). With efficient technologies developed, we will uncover the transcriptome-wide distribution of RNA modifications and study their role in tumour development. In addition, we will explore the potential clinical application of RNA modifications in the circulating cell-free RNA for non-invasive cancer diagnostics.

Disease Relevance

Cancer.

Key Technology

Chemical biology, sequencing technologies.

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

- 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.
- 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
- 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
- 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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1.7 Engineering human lectins to decipher the glycodynamics impairing T-cell immunity in cancer

Dr Pablo Cespedes & Dr Ricardo Fernandes

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Project Overview

The Background: The Cespedes-Donoso lab is focused in understanding the molecular factors influencing T-cell fitness and function in disease. We will focus on identifying the post-translational modification (PTMs) enzymes modulating the dynamics of receptor-ligand interactions at different length and time scales, from minutes of receptor binding within model immune synapses to the tracking of cell-cell communication unfolding within hours and days in lymphoid organoids and cancer-affected peripheral tissues. Our group has identified a number of enzymes differentially expressed in human T cells and we are currently performing loss-of-function experiments to identify those PTM enzymes regulating immune receptor trafficking, binding to cognate ligands and shedding into effector extracellular particles. However, whether antigen-presenting cells' PTM enzymes influence the trafficking, presentation, recycling and clustering of ligands promoting tumor rejection is little studied and understood. Glycosyltransferases, and more importantly, sialyltransferases and neuraminidases are enzymes suspected to influence the response of immune cells to tumors. For example, hyper-sialylation is commonly observed in cancers and correlates with poor immune and clinical outcomes, however, the biochemical, biophysical and metabolic mechanism underlying these observations are elusive.

The Challenge: Human lectins are carbohydrate binding proteins with a known low affinity for cognate ligands. Thanks to the secondary supervision by, and collaboration with Ricardo Fernandes we plan to develop recombinant lectins and lectin-derived nanobodies with increase avidity for human sugars. These novel lectins will complement our already validated panels for sugar profiling using both multi-dimensional flow cytometry and fluorescence microscopy, enabling the unprecedented characterization of tumor and immune glycobiology and within multiple human tissues.

Disease Relevance

Our goal is to perform a comprehensive profiling of key monosaccharides and glycans mediating either tumor immune escape or promoting T-cell-mediated killing. This project has the potential to identify novel glycans genitively modulating tumor immunity and offers the opportunity to reveal pharmacological targets and the development of novel detection nanobodies and biologics that can be repurposed for cancer therapy.

Key Technology

Protein engineering, Tissue Imaging, Super-resolution Microscopy, Airyscan Microscopy, High-dimensional flow cytometry, tissue and cell proteomics, transcriptomics and data analysis.

Training Opportunities

In all abovementioned methods. Students will be trained in protein engineering, tissue processing for high-content imaging, and the development and application of a number of semi-synthetic methods for the study of T-cell communication (immune synapses) at different time and length scales, including synthetic antigen presenting cells (in the form of bead supported lipid bilayers -BSLBs), glass-supported lipid bilayers for synapse imaging, and lymphoid organoids for the study of complex intercellular networks relevant for tumorigenesis and cancer progression[1-4].

Key Publications

- Cespedes, P. F., Beckers, D., Dustin, M. L. & Sezgin, E. Model membrane systems to reconstitute immune cell signaling. *FEBS J* **288**, 1070-1090 (2021). https://doi.org:10.1111/febs.15488
- Cespedes, P. F. & Dustin, M. L. Preparation of Bead-supported Lipid Bilayers to Study the Particulate Output of T Cell Immune Synapses. *J Vis Exp* (2022). https://doi.org:10.3791/63130
- Saliba, D. G. *et al.* Composition and structure of synaptic ectosomes exporting antigen receptor linked to functional CD40 ligand from helper T cells. *Elife* **8** (2019). https://doi.org:10.7554/eLife.47528

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

1.8 Modulating inhibitory receptor signalling to enhance the T cell response in cancer

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Project Overview

Abstract

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 project aims to dissect ligand-driven and ligand-independent IR signalling processes to gain insights into the IR signalling mechanism. This will be achieved by determining the potency of IR signalling for a group of receptors, identifying direct mediators of IR signalling, and developing a phosphatase-mediated approach to inhibit IR signalling. This proposal seeks to deepen our understanding of IR signalling and generate novel insights 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)[1]. In this approach, the phosphatase domain of CD45 acts intracellularly, in *cis,* on the p-Tyr residues of the PD-1 signalling motif, thus inhibiting sustained signalling. We have shown that *Receptor Inhibition by Phosphatase Recruitment* (RIPR), potentiates T cell activity beyond that seen with PD-1/PD-L1 antagonist antibodies, both in the presence and absence of PD-1 ligand-binding *in vitro*, and reduces tumour growth in mouse models[1].

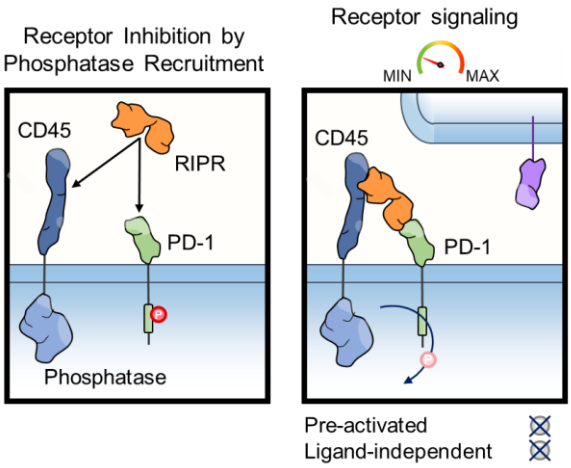

Fig. 1 | Receptor Inhibition by Phosphatase Recruitment. RIPR combines checkpoint blockade with CD45 recruitment. CD45 recruitment dephosphorylates receptors such as PD-1.

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. These distinct domains are likely to affect the IR signalling potency. To test this, we will determine the relative IR potencies by establishing an IR signalling platform in primary CD4+ and CD8+ T cells. 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: Identification of early and late mediators of IR signalling

We will determine the direct signalling mediators to IR signalling using targeted protein pull-down strategies complemented by mass spectrometry (MS)-based proteomics (in collaboration with Dr Adan Pinto-Fernandez's Group) and epigenetic profiling (in collaboration with Dr. Chunxiao Song's Group). Moreover, we will map the phosphorylation

status of the inhibitory motifs and protein-complex composition by western blot and optimised affinity purification. Next, we will determine the contribution of specific mediators and transcription factors by deleting a single or a combination of targets using CRISPR/Cas9. Collectively, this approach aims to identify early and late signalling mediators of various IRs and their role in suppressing T cell responses.

Aim 3: 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. Newly generated molecules will be characterised in biophysical and in vitro stimulation assays. Binding on-rate, off-rate and affinity will be determined by surface plasmon resonance. After characterising the binding properties of the RIPR molecules, their ability to potentiate T cell cytotoxic functions will be determined *in vitro* using co-culture assays with T cells and target cells. Markers of T cell activation will be quantified longitudinally using flow cytometry, western blotting and ELISAs. This comprehensive approach is expected to identify determinants of RIPR activity for various checkpoint receptors. This information will be used to guide the design of future antagonists of checkpoint receptor signalling with strong potential for therapeutic applications.

Summary of milestones and expected outcomes:

(i) Obtaining a comparative list of signalling potencies for all tested IRs;

(iii) identification of early and late signalling mediators of IRs;

(iv) Providing proof-of-concept for inhibiting IR signalling by phosphatase recruitment;

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; mass-spectrometry and epigenetic analysis of functional T cell states.

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

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

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

Dr Ricardo Fernandes & Prof. Tao Dong

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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 (> 10^9) peptide-MHC libraries to be displayed at the surface of yeast cells, after which we will use an affinity-based screen to identify peptides recognised by TCRs of interest. This affinity-based approach will be complemented by a functional screen using an engineered system in mammalian cells. In this recently developed approach, the peptide-MHC library is fused to a CAR-like signalling module and displayed in T cells. This functional-based selection hijacks the unique sensitivity and specificity of the CD28/CD3 signalling modules to report on a productive TCR/pMHC interaction. Sorting of cells based on the upregulation of activation markers such as CD69 and CD25 will be used to isolate agonist peptides of different potency.

The combination of affinity- and activity-based selections will guide the identification of potent agonist mimotopes, self-peptides or neoantigens using custom-built algorithms to rank closely related wild-type peptides. The identification of peptides recognised by tumour-reactive T cells will facilitate their expansion and detection using peptide-MHC molecules. Moreover, following isolation or activation with agonist peptides, tumour-reactive T cells will be characterised using single-cell transcriptomics and proteomics, for 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.

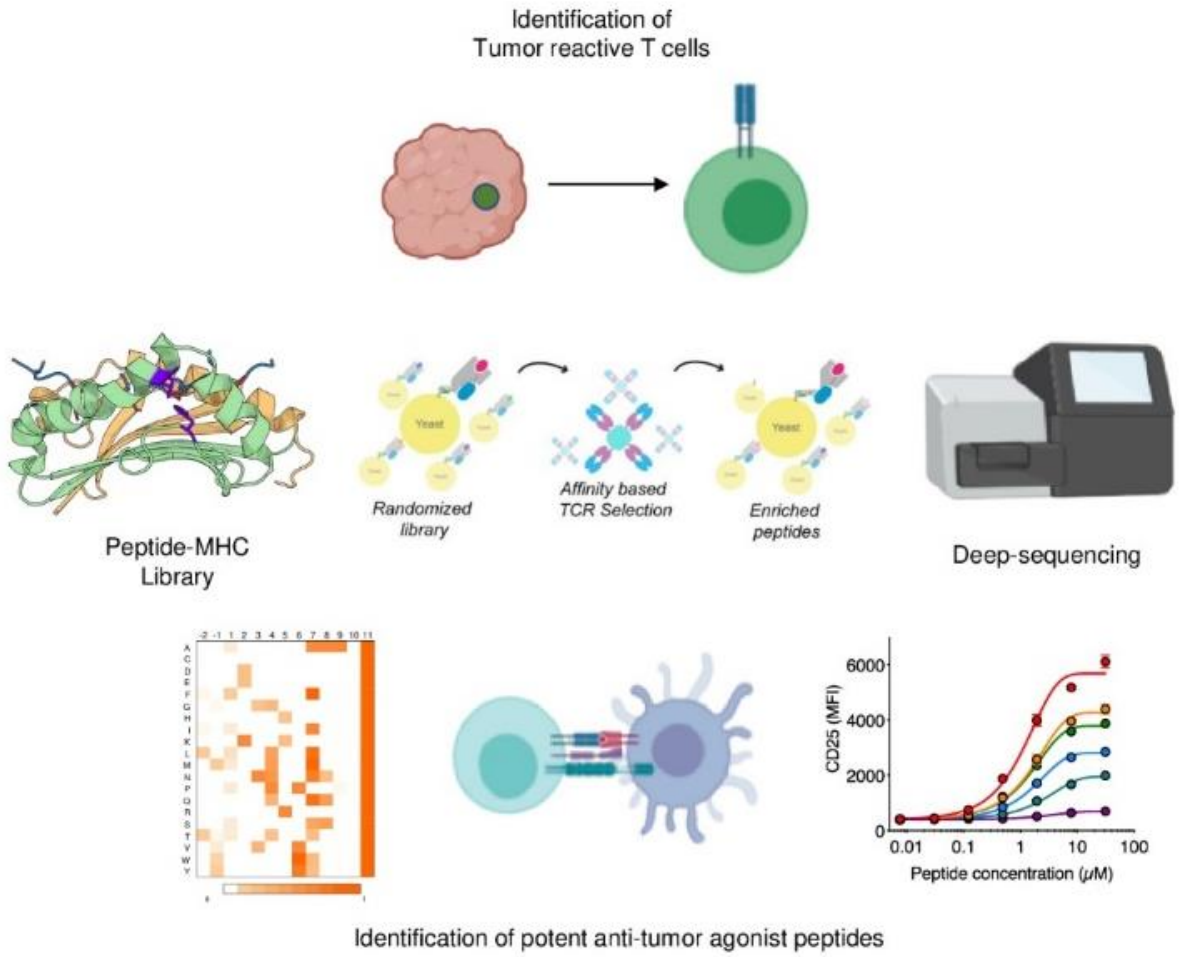

Figure 1. General overview of the experimental approach to discover peptide antigens to elicit robust anti-tumor T cell responses.

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; biophysical analysis of protein-protein interactions; T cell activation.

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

- 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.

- 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.
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- 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.

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

Prof. Yang Shi & Prof. Stepan Constantinescu

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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), which still presents 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

- 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
- 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.
- Cell Rep. 2021, Nov 9;37(6):109967. doi: 10.1016/j.celrep.2021.109967.

2. Viral Infection Theme

2.1 SARS-CoV-2 replication, assembly, and egress

Prof. Peijun Zhang

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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, cryo-electron tomography, in situ structural biology, cryoFIB/SEM

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

- 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, in press*, bioRxiv, 2023 May 24.
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2.2 Imaging HIV-1 nuclear import by in situ cryo-tomography and correlative microscopy

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Project Overview

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

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

Disease Relevance

HIV-1, retrovirus infection

Key Technology

cryoEM, cryo-electron tomography, in situ structural biology, cryoFIB/SEM

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

- 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
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by Cryo-electron Microscopy and All-atom Molecular Dynamics. *Nature* 497(7451):643-6. Featured on the cover of Nature.

2.3 Structural and functional characterisation of the influenza virus transcriptase

Prof. Ervin Fodor

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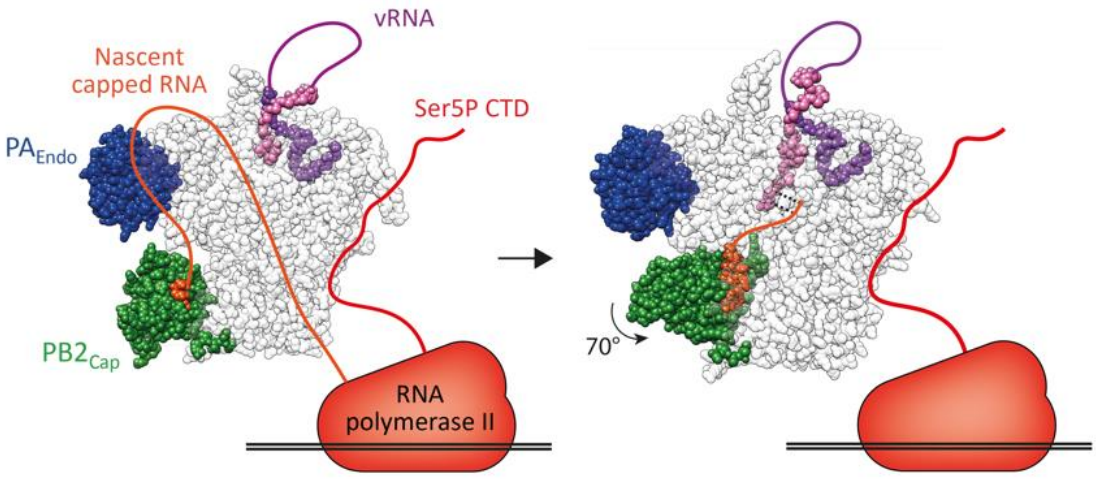
Project Overview

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

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



The aim of this project is to characterise the influenza virus transcriptase structurally and functionally. To transcribe viral genes the influenza virus polymerase associates with host RNA polymerase II (Pol II). This interaction enables the viral polymerase to access nascent capped RNA and cleave it to generate capped RNA fragments that it uses to prime viral transcription. Here we aim to identify key molecular interactions between the influenza virus polymerase and the Pol II complex using a combination of cross-linking mass spectrometry

(XL-MS) and proximity labelling techniques. The role of identified factors during the transcription of viral genes will be evaluated using Crispr-knockout and gene silencing in infection experiments using wild-type and mutant influenza viruses, generated by using reverse genetics. Host factors directly associating with the influenza virus transcriptase will be further evaluated using structural methods such as cryo-electron microscopy and x-ray crystallography.

Disease Relevance

Viral infections – influenza

Key Technology

Mass spectrometry, Crispr and gene silencing, reverse genetics, cryo-EM, crystallography

Training Opportunities

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

Key Publications

- Zhu Z, Fodor E, Keown JR (2023) A structural understanding of influenza virus genome replication. *Trends Microbiol* 31(3):308-319
- te Velthuis AJW, Grimes JM, Fodor E (2021) Structural insights into negative strand RNA virus RNA polymerases**.** *Nat Rev Microbiol* 19(5):303-318.
- Carrique L, Fan H, Walker AP, Keown JR, Sharps J, Staller E, Barclay WS, Fodor E, Grimes JM (2020) Host ANP32A mediates the assembly of the influenza virus replicase. *Nature* 587(7835):638-643.
- Fan H, Walker AP, Carrique L, Keown JR, Serna Martin I, Karia D, Sharps J, Hengrung N, Pardon E, Steyaert J, Grimes JM and Fodor E (2019) Structures of influenza A virus RNA polymerase offer insight into viral genome replication. *Nature* 573(7773):287-290.

- Serna Martin I, Hengrung N, Renner M, Sharps J, Martínez-Alonso M, Masiulis S, Grimes JM, Fodor E (2018) A Mechanism for the Activation of the Influenza Virus Transcriptase. *Mol Cell* 70:1101-1110.

2.4 Characterizing the ultrastructure SARS-CoV-2 infected cells using in-situ cryo-Electron Tomography

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

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Project Overview

SARS-CoV-2 has caused one of the greatest global health challenges to date and there is an urgent need to understand the fundamental mechanisms of virus replication. 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. Cryo-EM imaging of the SARS-CoV-2 life cycle has shown that viral replication is spatially well-organised with each stage taking place in dedicated interdependent cytoplasmic compartments. The aim of this project is to explore the fundamental aspects of sub-cellular compartmentalisation of viral infection focusing on the structural composition of the replicase complex. Using the world-leading cryo-EM bio-imaging capabilities of the oxford particle imaging centre (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.

Disease Relevance

SARS-CoV-2, COVID19, Respiratory infections

Key Technology

Cryo-EM, Focused Ion Beam (FIB) milling, Electron tomography, correlative light and electron microscopy.

Training Opportunities

Students will be fully trained in all aspects of in-situ cryo-electron tomography, molecular virology and working in containment level 3 environment. Further significant bioinformatic training will be provided to analyse the wealth of data generated.

Key Publications

- Georg Wolff *et al.* A molecular pore spans the double membrane of the coronavirus replication organelle. *Science* (2020).
- Zimmermann *et al.* SARS-CoV-2 nsp3-4 suffice to form a pore shaping replication organelles. *BioRxiv* (2022)
- Wing, P.A.C.*, et al.* Hypoxic and pharmacological activation of HIFs inhibits SARS-CoV-2 infection of lung epithelial cells. *Cell Reports* (2021).

2.5 Understanding the cellular factors governing BK virus replication in kidneys to identify novel treatment approaches

Dr Peter Wing, Dr Matthew Bottomley & Prof. David Mole

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Project Overview

BK polyoma virus (BKpyV) is a prevalent small dsDNA polyomavirus infecting the renal tubule and can cause severe complications in renal transplant patients resulting in inflammation within the graft (BK nephropathy), transplant rejection or haemorrhagic cystitis in the short-term, and urothelial cancer with chronic infection. There are limited treatment options and the cellular determinants governing reactivation of BKpyV in kidney transplant patients remain undefined. The microenvironment in the renal tubule often experiences a significantly lower oxygen tension compared to other organs. The cellular response to low oxygen is governed by the hypoxia inducible factor (HIF) signalling axis, which orchestrates a global transcriptional response to promote pathways enabling a cell to adapt to a hypoxic environment. HIF signalling may define BKV tropism as it exhibits transient replication in the airway with high oxygen levels and persistent replication in the renal tubules with low oxygen levels. Recent work has shown that HIF activation promotes BKpyV gene promoters and some studies suggest that tumour hypoxia and aberrant HIF signalling may drive BKpyV oncogenesis in bladder cancers arising in solid organ transplant recipients.

The aim of this project is to define the cellular determinants governing BKpyV replication and reactivation in cellular and organoid models of replication. Using a multi-disciplinary approach of molecular virology, hypoxia biology and spatial transcriptomics available in the Wing, Bottomley and Mole labs, this work will define the interplay between BKpyV replication and the cellular hypoxic response using a range of techniques including spatial transcriptomic analysis of existing patient kidney transplant biopsies. The results of this research may have implications for the development of novel therapeutic interventions to address a serious clinical problem that affects one in five kidney transplant recipients and lacks any specific treatment currently.

Disease Relevance

Renal medicine, kidney transplantation, BKpyV infection

Key Technology

Virology, molecular biology, Single cell and bulk-RNAseq, spatial transcriptomics, organoid cultures.

Training Opportunities

Students will be fully trained in techniques involved in viral propagation and molecular biology as well as bioinformatic analysis of bulk, single cell RNA sequencing and spatial transcriptomic data sets. Techniques include, but are not limited to:

- Multilaser flow cytometry
- Viral culture
- Establishing primary polarised renal proximal tubule epithelial cells for infection experiments
- Imaging viral infeciton using super-resolution confocal microscopy
- Histological preparation and analysis of human tissue sections (multiplex immunohistochemistry, immunofluorescence)
- RNA extraction and gene expression analysis (using RT-PCR and hybridisation-based approaches)
- Bioinformatic analysis of high-plex spatial transcriptomic data

Key Publications

- Signorini, L., Croci, M., Boldorini, R., *et al.,* 2016. Interaction Between Human Polyomavirus BK and Hypoxia Inducible Factor-1 alpha. Journal of Cellular Physiology 231, 1343–1349. https://doi.org/10.1002/jcp.25238
- Wing, P.A.C., Keeley, T.P., Zhuang, X., Lee, J.Y., Prange-Barczynska, M., Tsukuda, S., Morgan, S.B., *et al.,* 2021. Hypoxic and pharmacological activation of HIF inhibits SARS-CoV-2 infection of lung epithelial cells. Cell Rep 35, 109020. https://doi.org/10.1016/j.celrep.2021.109020
- Cross A.R., Gartner L., *et al.* 2023. Opportunities for high-plex spatial transcriptomics in solid organ transplantation. Transplantation (ePub ahead of print). https://doi.org/10.1097/TP.0000000000004587

2.6 Examining the Interplay between Hypoxia Signalling, Viral Replication, and Immune Cells in Respiratory Viral Diseases

Prof. Jane McKeating & Dr Peter Wing

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Project Overview

Respiratory infections in humans are responsible for a significant proportion of global deaths, approximately 4.25 million/year, mostly in children and older adults. The COVID-19 pandemic highlighted the importance of understanding fundamental host processes that viruses exploit to infect the respiratory tract. Respiratory syncytial virus (RSV) is the leading cause of infant hospitalisation worldwide, infecting approximately 34 million children each year and is an increasingly recognised cause of morbidity and mortality in the elderly and immunosuppressed adults. Although RSV infection is generally confined to the respiratory epithelium the resulting neutrophilic lung inflammation can be life-threatening. The only approved prophylactic treatment is Palivizumab (neutralising anti-RSV Fusion antibody), highlighting the urgent need for antiviral agents.

The cellular microenvironment is constantly in flux, and to maintain homeostatic balance, cells must rapidly adapt to variations in their local surroundings. A key element that governs the cellular state is oxygen availability, where Hypoxia Inducible Factors (HIFs) activate the transcription of genes regulating cell metabolism and immune defences. We previously reported that HIFs inhibit SARS-CoV-2 infection and pathology and recent data show their role in suppressing RSV infection. This project will use state-of-the-art RSV and SARS-CoV-2 replication systems, as well as animal models, to examine the interplay between HIFs, viral replication, and immune cells, with a focus on understanding their spatial relationship in respiratory tissue. We are keen to translate data from our experimental model systems to a clinical setting and study the relationship between HIFs, immature neutrophils, and T cells in infected clinical samples using mass cytometry imaging methods. Comparative analysis of transcriptomic and immunotyping data from experimental model systems with human clinical data provides an opportunity to validate HIF regulation of these fundamental immune regulatory pathways, which will inform the discovery of antiviral therapies for the treatment of respiratory viral diseases.

Disease Relevance

Hypoxia, Virology, SARS-CoV-2, RSV, viral pneumonia, respiratory disease

Key Technology

Students will be fully trained in techniques involved in viral propagation and molecular biology as well as bioinformatic analysis of bulk, single cell RNA sequencing and spatial transcriptomic data sets. Techniques include, but are not limited to:

- Viral culture
- Establishing air-liquid interface cultures of the airway epithelium ells for infection experiments
- Imaging viral infection using super-resolution confocal microscopy
- Histological preparation and analysis of human tissue sections (multiplex immunohistochemistry, immunofluorescence)
- RNA extraction and gene expression analysis (using RT-PCR and hybridisation-based approaches)
- Bioinformatic analysis of high-plex spatial transcriptomic data

Training Opportunities

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

Key Publications

- Sun *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,* doi : 10.1126/sciimmunol.abl8881
- Wing *et al* 2022. Hypoxia inducible factors regulate infectious SARS-CoV-2, epithelial damage and respiratory symptoms in a hamster COVID-19 model. *PLoS Pathogens*. doi: 10.1371/journal.ppat.1010807
- Lee *et al* 2022. Absolute quantitation of individual SARS-CoV-2 RNA molecules provides a new paradigm for infection dynamics and variant differences. *eLIFE,* doi: 10.7554/eLife.74153
- Wing *et al* 2021. Hypoxic and pharmacological activation of HIF inhibits SARS-CoV-2 infection of lung epithelial cells. *Cell Rep.* doi: 10.1016/j.celrep.2021.109020.
- Zhuang X *et al* 2021. The circadian clock component BMAL1 regulates SARS-CoV-2 entry and replication in lung epithelial cells. *iScience* doi : 10.1016/j.isci.2021.103144

2.7 Understanding the host pathways that define hepatitis B virus persistence

Prof. Jane McKeating, Dr James Harris & Dr Peter Wing

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Project Overview

Background: Chronic hepatitis B is one of the world's unconquered infections and presents as a spectrum of liver disease, reflecting a dynamic interaction between the virus and immune system. Current treatments only suppress hepatitis B virus (HBV) replication and are not curative and there is an urgent need for new therapies. Defining the host pathways that regulate HBV DNA persistence and transcriptional activity will inform the development of new curative treatments.

HBV replication varies spatially within the liver, reflecting localised hepatocyte-intrinsic and liver-resident immune resistance mechanisms. We have developed sensitive methods to visualise HBV RNA molecules in liver biopsy samples and to study the behaviour of individual hepatocytes. Combining this with spatial transcriptomics provides an integrated approach to study viral and immune parameters in the liver and a step-change in our understanding of this chronic disease.

Project aim: To define the HBV transcriptome at the single cell level in the chronic infected liver and to identify conserved pathways that regulate viral transcription.

Disease Relevance

Chronic hepatitis B is one of the world's most economically important diseases with a global burden 300 million infections. HBV replicates in the liver and chronic infection can lead to cirrhosis and hepatocellular carcinoma. This project seeks to understand the host pathways that regulate hepatocellular susceptibility to HBV infection and this knowledge will underpin the development of new curative therapies.

Key Technology

DPhil will utilise the following technologies:

- Viral replication model systems
- High resolution imaging of HBV and host transcripts in liver tissue.
- AI informed analysis and quantitation of viral transcriptome and 3D reconstruction.
- Long-read sequence analysis of HBV RNAs.

Training Opportunities

The student will join a dynamic and lively team of biologists funded by a Wellcome Discovery Award that will provide a unique training environment to gain expertise in super resolution imaging techniques to visualize viral RNAs in complex tissues, digital spatial profiling and bio-informatic analysis of inflammatory transcriptomic data sets.

Transferable skills include oral presentations at lab meetings, critical review of published literature at journal clubs and scientific writing by reviewing and drafting manuscripts for publication. The student will work in Nuffield Department of Medicine Research Building 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

- Wing et al. 2021. Hypoxia inducible factors regulate hepatitis B virus replication by activating the basal core promoter. J Hepatology. doi 10.1016/j.jhep.2020.12.034.
- Schmidt et al. (2021). Targeting human Acyl-CoA:cholesterol acyltransferance as a dual virus and T cell metabolic checkpoint. Nature Comms doi: 10.1038/s41467-021-22967-7
- Zhuang et al (2021). Circadian control of hepatitis B virus replication. Nat Comms doi:10.1038/s41467-021-21821-0
- Lythgoe et al (2020). Estimation of cccDNA persistence during HBV infection using within-host evolutionary rates.Virus Evolution doi: 10.1093/ve/veaa063
- Wing et al (2019). (2019). A dual role for SAMDH1 in HBV cccDNA synthesis and RT-dependent particle genesis. Life Science Alliance doi : 10.26508/lsa.201900355

2.8 Evasion of innate immunity by monkeypox virus

Prof. Geoffrey L Smith

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Project Overview

This project will identify host proteins that restrict the replication of orthopoxviruss such as vaccinia virus and monkeypox virus and study their mechanisms of action. The project will also investigate virus countermeasures that antagonize these host factors and enable these 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 TRIM5, it has been demonstrated that these have anti-viral activity and are antagonized by viral counter-measures. Further, a detailed mechanistic study of TRIM5 and virus countermeasures led to the proposed re-purposing of existing drugs against monkeypox virus.

Disease Relevance

Relevant to the current widespread outbreak of monkeypox (mpox) caused by monkeypox virus.

Key Technology

Molecular virology, innate immunity, structural biology, proteomics

Training Opportunities

A training in a basic science project that includes virology, cell biology, innate immunity, proteomics and structural biology.

Key Publications

- 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.
- 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
- 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.
- Depierreux, D.M*., Altenburg,' A.F*., Soday, L., Fletcher-Etherington, A., Anthrobus, R., Ferguson, B.J., Weekes, M.P**. & Smith, G.L**. (2022). Selective modulation of cell surface proteins during vaccinia infection: a resource for identifying viral immune evasion strategies. *PLoS Pathogens* **18**, e1010612.
- 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.

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

Prof. Gavin Screaton & Ass.Prof. Juthathip Mongkolsapaya

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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 Sudan Ebolavirus, which caused a large outbreak in Uganda at the end of 2022, Nipah virus, and enterovirus.

Disease Relevance

When a new pathogen emerges and primarily results in a severe or deadly disease, it has the potential to trigger a significant outbreak or pandemic. This becomes particularly alarming when there are no available vaccines or drugs urgently needed to combat the disease.

Key Technology

Flow cytometer, single cell sorting, Molecular biology, gene editing, Cell culture, recombinant protein expression, reverse genetic/Gibson reaction to generate infection wildtype and mutated live viruses, and Structure Biology.

Training Opportunities

The student will join a team with over 20 years of experience in virology, immunology, and molecular biology. The student will receive training from and work closely with experienced post-docs in a wide range of techniques, for example virology (viral isolation, viral propagation, neutralization, and viral titration), generation of pseudovirus, immunology (ELISA, immunoprecipitation, SDS-PAGE, Western blot, FPLC and affinity purification, flow cytometry, single cell sorting, tissue/cell culture), and molecular biology (PCR, utilizing software programs to design primers, mutagenesis, deep sequencing of antibody repertoires, cloning, protein expression in bacterial, yeast, insect, and mammalian cell systems).

The student will also gain experience in generating monoclonal antibodies from single human and mouse B cells, using software and structural analysis to design new vaccines, and developing therapeutic or prophylactic agents. Additionally, the student will have the opportunity to collaborate closely with our partners in Oxford and both within and outside the UK.

Key Publications

- 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).

- 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).
- Rouvinski, A. *et al.* Recognition determinants of broadly neutralizing human antibodies against dengue viruses. *Nature* **520**, 109-113, doi:10.1038/nature14130 (2015).
- 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).
- Dejnirattisai, W. *et al.* Cross-reacting antibodies enhance dengue virus infection in humans. *Science* **328**, 745-748, doi:10.1126/science.1185181 (2010).

3. Other Themes

3.1 Establishing clinically relevant disease subtypes in severe infection to advance personalised medicine

Prof. Julian Knight

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Project Overview

This project aims to understand the nature of individual variation in the response to infection that leads to severe disease, the role of disease subtypes, how these may arise, and be taken forward into clinical practice to improve patient care.

We have shown that white blood cell transcriptomic signatures predict underlying response state, outcome and response to therapy in sepsis. We have discovered a specific sepsis endotype associated with poor outcome. The likelihood of this endotype in an individual patient at a given time of assessment can be quantified through a transcriptomic immune dysfunction response score, with applicability across different infectious aetiologies including SARS-CoV-2. We found that this endotype arises due to specific immature neutrophil populations that are dysfunctional and altered emergency granulopoiesis.

In COVID-19, we demonstrated that multi-omic profiling in blood allows an integrated systems biology approach to understanding the nature and basis of observed disease heterogeneity and drivers of severe illness, shared and specific to COVID-19, sepsis and influenza (COVID-19 Multi-omic Blood ATlas Consortium).

This project will aim to build on these findings to further characterise the maladaptive response to infection, including the existence and nature of additional disease endotypes and treatable traits. This will leverage ongoing work in sepsis and COVID-19, cutting-edge analytical tools, and deep clinical phenotyping. The work will explore how to define informative biomarkers relevant to point of care testing, clinical utility and opportunities to develop targeted therapy. We will also investigate relevance in non-infectious critical illness. The project will benefit from access to large genomic and clinical datasets, both publicly available and those generated in house.

Disease Relevance

The dysregulated host response to infection results in organ dysfunction and death, accounting for substantial morbidity and mortality including 11 million deaths globally each year from sepsis, as well as to deaths from specific infections such as SARS-CoV-2. How and why this maladaptive response occurs in specific individuals with an infection remains unclear. This DPhil project will aim to investigate this in sepsis and COVID-19, with a view to developing personalised therapy that is appropriate to the individual patient at a particular stage in their illness, including in convalescence.

Key Technology

These will include bioinformatics to analyse -omic and other multi-modal high dimensional data, including systems biology and integrative analysis approaches to maximise the informativeness of such datasets for endotype discovery and validation; work with clinical samples and datasets; development of biomarkers; application of single cell -omic and immune profiling approaches to further define mechanism and basis of pathogenesis.

Training Opportunities

You will join an experienced team of clinicians and scientists who have made significant progress in this area, ensuring the work is tractable and state of the art. The Knight and Mentzer groups have pioneered work into the genetics and genomics of immunity and infectious disease susceptibility.

This project will offer a comprehensive training programme in translational genomic science and its clinical application, together with bioinformatics, molecular biology and immunology using state-of-the-art facilities for such research.

There are established sample and data collections for the proposed work, together with a very strong collaborative research network with other researchers in this area. The required wet lab and bioinformatic approaches are well established with an expert and supportive team of postdoctoral researchers as part of this programme of work in the groups.

Specific training will include

- bioinformatics, machine learning and integrative systems biology to leverage genomic, epigenomic and metagenomic data together with deep clinical phenotyping including integration of diverse multimodal data types, identification of patient and feature clusters as well as potential drug targets

- single cell multi-omics including using the 10X Chromium and BD Rhapsody systems
- immune profiling using cutting-edge immunological assays including flow and mass cytometry

Students will benefit from working within a supportive research environment with supervisors who have a strong track record in graduate student training and mentoring. You will have the opportunity to regularly present your work within the groups, to your peers within the Centre as well as at international conferences. Students are encouraged and supported to undertake further relevant training courses in Oxford and elsewhere depending on need.

Key Publications

- Cano-Gamez E, Burnham KL, Goh C, Allcock A, Malick ZH, Overend L, Kwok A, Smith DA, Peters-Sengers H, Antcliffe D, Investigators GA, McKechnie S, Scicluna BP, Van Der Poll T, Gordon AC, Hinds CJ, Davenport EE, Knight JC (2022). An immune dysfunction score for stratification of patients with acute infection based on whole-blood gene expression. *Science Translational Medicine* **14** eabq4433
- COMBAT Consortium (2022). A blood atlas of COVID-19 defines hallmarks of disease severity and specificity. *Cell* **185** 916-938
- Davenport EE, Burnham KL, Radhakrishnan J, Humburg P, Hutton P, Mills TC, Rautanen A, Gordon AC, Garrard C, Hill AV, Hinds CJ & Knight JC (2016). Genomic landscape of the individual host response and outcomes in sepsis: a prospective cohort study. *Lancet Resp Med* **4** 259-71
- Kwok AJ, Allcock A, Ferreira RC, Cano-Gamez E, Smee M, Burnham KL, Zurke YX, McKechnie S, Mentzer AJ, Monaco C, Udalova IA, Hinds CJ, Todd JA, Davenport EE & Knight JC (2023). Neutrophils and emergency granulopoiesis drive immune suppression and an extreme response endotype during sepsis. *Nature Immunology* **24** 767-779
- Kwok AJ, Mentzer A & Knight JC (2021). Host genetics and infectious disease: new tools, insights and translational opportunities. *Nature Reviews Genetics* **22** 137-153

3.2 A multi-omic approach to understand the immune response to pleural infection in a disease context

Dr. Nikolaos Kanellakis & Prof. Julian Knight

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Project Overview

Summary: This cutting-edge project is part of the Oxford Study of Pleural Infection with Microenvironment Phenotyping, a translational, unbiased, single cell, multicompartment, and multi-omics study to elucidate pleural infection pathogenesis, progression, and immune response through ex vivo phenotyping. The work is a collaboration between the Laboratory of Respiratory Research (Dr Kanellakis) and the Functional Genomics of Immunity research group (Prof Knight).

Background: The lungs are lined by a double layer of mesothelial cells. The area in-between these two membranes is called the pleural cavity. Pleural Infection is a common and complex disease where bacteria invade the pleural space and cause accumulation of fluid known as pleural effusion.[1] Epidemiological data shows increasing incidence of pleural infection worldwide, especially in the elderly.[2-4]

Our previous work: In a previous study we investigated the microbiology of pleural infection. For this, we applied 16S rRNA sequencing on pleural fluids specimens collected for the largest prospective study on pleural infection (PILOT study, n=243)[13]. Pleural infection was predominately polymicrobial with diverse bacterial frequencies observed in monomicrobial and polymicrobial disease (Figure 1).[14] The presence of anaerobes or bacteria of the *Streptococcus anginosus* group was associated with better patient survival, whereas the presence or dominance of *Staphylococcus aureus* was linked with lower survival.[14]

The clinical problem: Clinical outcomes of pleural infection remain poor despite treatment.[1,5-8] Recent studies demonstrate mortality of 20%, rising to 40% in vulnerable populations such as the elderly.[9] Pleural infection usually develops as a complication of pneumonia.[10-12] The exact molecular pathways of disease progression remain unknown and therefore pleural infection remains a significant clinical problem.

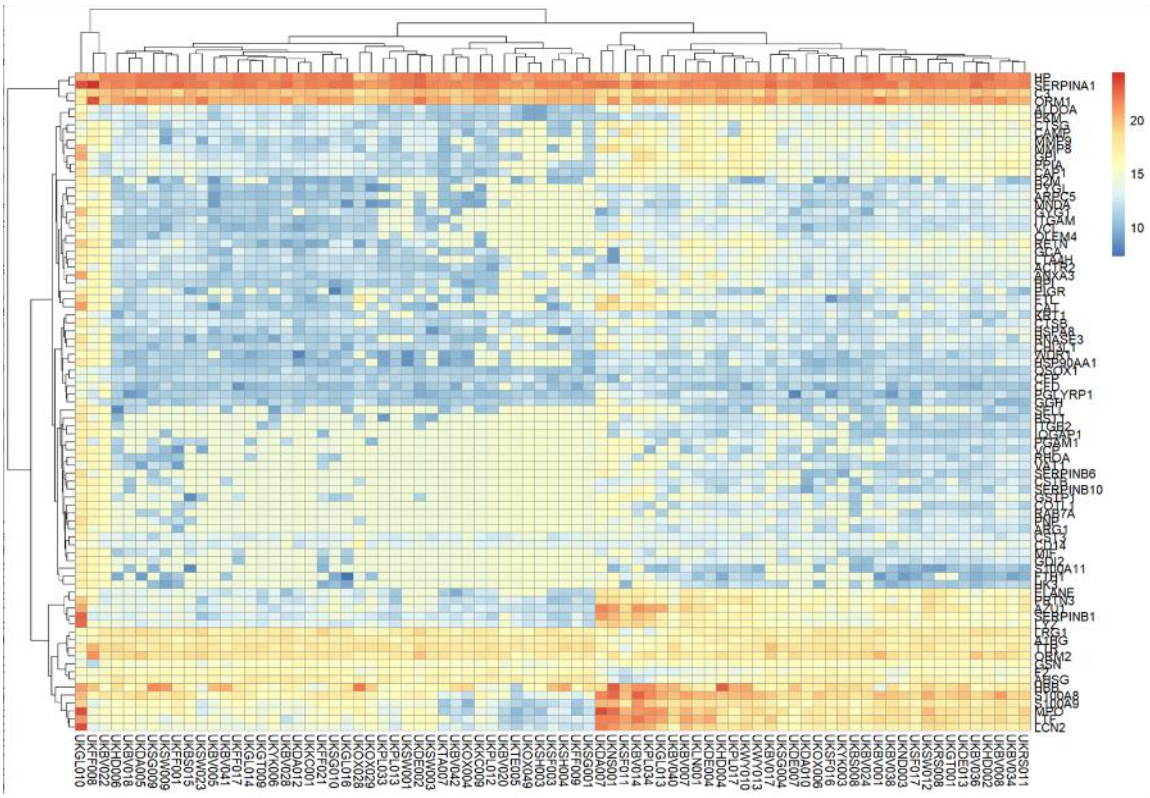

Figure 1. Heatmap of unsupervised hierarchical clustering using the Euclidean distance and the complete-linkage method. The samples are clustered based on the expression of 84 proteins associated with neutrophil mediated immunity. Each row represents a protein and each column a sample. There are two major clusters with diverse protein expression. Deep blue denotes low expression whereas far red high expression.

Aim: This is a project with the aim to ex vivo phenotype and functionally characterise immune cells derived from pleural fluid, blood, and pleural biopsy specimens from pleural infection patients.

Experimental Plan: Identify cell specific gene and chromatin accessibility signatures. Single cell RNA sequencing (BD Rhapsody) and cell surface protein profiling (BD AbSeq) tagging common immune markers will be applied to pleural fluid and whole blood cells to elucidate the pleural fluid microenvironment. Moreover, single cell transposase-accessible chromatin (ATAC-seq, 10X Genomics) sequencing (NovaSeq Illumina) will be used to identify differences at the chromatin patterns. Clinical specimens from patients with a pleural effusion due to heart failure and cancer would be used as controls. In addition to samples, we will collect radiological (CT, ultrasound) and clinical data including length of hospital stay, requirement for further pleural interventions (drain insertion, surgery, intensive care) death, functional status (lung function, exercise ability).

The pleural fluid and blood/serum specimens would be subjected to label-free mass spectrometry to profile the proteome.

The multilayer approach would allow us to deeply phenotype pleural infection. We will integrate the data (single cell transcriptomic, ATAC, and proteomic) to investigate the differences in intrapleural immune and fibrinolytic microenvironments. Moreover, we will identify and annotate immune cell populations, their biological signatures and key mediators of response. We will relate findings to the time course of disease, define predictive biomarkers of response states and validate potential targets using genome editing and related approaches.

Disease Relevance

Despite advances in medical diagnostic and therapeutic strategies, pleural infection (empyema or complex parapneumonic effusion) is an important problem worldwide that continues to be associated with substantial morbidity and mortality. The incidence of pleural infection in both adult and paediatric populations continues to rise. Pleural infection is a complex, complicated, and severe disease with a severe clinical impact. The mechanisms of progression and immune responses are poorly understood. One in five patients will need surgical intervention to adequately treat their pleural infection. There is in unmet clinical need to better understand the pathophysiology of pleural infection. This clinical translational project aims to elucidate the pleural infection microenvironment with a view to develop better clinical management and improve clinical outcomes.

This project could lead to the discovery of novel biomarkers to stratify pleural infection patients to the most appropriate treatment. The outcomes of this project could lead to a clinical trial.

Key Technology

This is a clinical translational project which involves the latest cutting-edge laboratory methods including: Single cell RNA sequencing and transposase-accessible chromatin sequencing (ATAC-Seq). The BD Rhapsody and 10X genomics platforms would be applied. Label-free mass spectrometry would be used to profile the proteome.

Training Opportunities

This is a clinical translational project. The DPhil student would get involved with both translational and clinical research. Moreover, the DPhil student will have the opportunity to present their work in international conferences and publish in peer reviewed journals. Moreover, the student would be trained in the latest technologies of single cell RNA sequencing and transposase-accessible chromatin sequencing (ATAC-Seq). Moreover, the student would get involved with mass spectrometry and bioinformatic analyses.

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

Key Publications

- Cano-Gamez E, Burnham KL, Goh C, Malick ZH, Kwok A, Smith DA, Peters-Sengers H, Antcliffe D, Investigators G, McKechnie S, Scicluna BP, van der Poll T, Gordon AC, Hinds CJ, Davenport EE & Knight JC (2022). An immune dysfunction score for stratification of patients with acute infection based on whole blood gene expression. *Science Translational Medicine* 10.1126/scitranslmed.abq4433
- COMBAT Consortium† (2022). A blood atlas of COVID-19 defines hallmarks of disease severity and specificity. *Cell* **185**, 916-938 e958
- NI Kanellakis et al., The bacteriology of pleural infection (TORPIDS): an exploratory metagenomics analysis through next generation sequencing, The lancet Microbe, DOI: https://doi.org/10.1016/S2666-5247(21)00327-X
- JP Corcoran at al., Prospective validation of the RAPID clinical risk prediction score in adult patients with pleural infection: the PILOT study, Eur Respir J., DOI: 10.1183/13993003.00130-2020
- E. Bedawi, ERS/ESTS statement on the management of pleural infection in adults Eur Respir J., DOI: 10.1183/13993003.01062-2022

3.3 Investigation of heterogeneity in Type-2 cells and their roles in human airway diseases

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Project Overview

Type-2 inflammation is the hallmark of multiple allergic diseases including allergic/eosinophilic asthma, which is characterized by elevated eosinophils and type-2 cytokines. Type-2 cells are the key drivers of type-2 inflammation, among which eosinophils and type-2 cytokine producing T cells (T2 cells) are the essential effector cells. Eosinophilic asthma is a heterogeneous disease with distinct clinical phenotypes, for example severe eosinophilic asthma with poor response to steroids or biologics or with worse lung function and susceptible to airway remodelling, which might be mediated by functionally different type-2 cell subgroups. However, the heterogeneity in human type-2 cells and their mechanisms in driving airway inflammation and relationship with different phenotypes are not fully elucidated. Therefore, the aims of this project lie on the 1) dissecting the T2 cells and eosinophil subgroups; 2) understanding the functional diversity and their mechanisms of T2 cells and eosinophils subgroups; 3) investigating the relationship between T2 cells and eosinophils subgroups and different clinical phenotypes in patients with asthma. This project will address:

Aim 1: Identification of T2 cell and eosinophil subgroups.

Confirm the heterogeneity of T2 cells and eosinophils by clustering analysis of their transcriptome profiles. Screen surface markers to identify and dissect T2 cell and eosinophil subgroups.

Aim 2: Investigation of functions of T2 cells and eosinophil subgroups in airway inflammation.

Study the biological and pathological functions of T2 cell and eosinophil subgroups in airway inflammation. Investigate the mechanisms of the diverse functions including the signalling pathways.

Aim 3: Investigation of the relationship between T2 cell and eosinophil subgroups and asthma with different clinical features.

Compare T2 cell and eosinophil subgroups among different phenotypic asthma and treatment responses. Compare T2 cell and eosinophil subgroups before and after different treatments.

Disease Relevance

Asthma, COPD

Key Technology

Multiparametric FACS, FACS sorting, primary cell culture, cell cloning, and bioinformatics

Training Opportunities

Multiple lab techniques/skills, bioinformatics, data analysis and presentation, seminars, etc.

Key Publications

- Luo J, et al. Resistance to apoptosis underpins the corticosteroid insensitivity of group 2 innate lymphoid cells. J Allergy Clin Immunol. 2019 Dec;144(6):1722-1726.e10.
- Hilvering B, et al. Synergistic activation of pro-inflammatory type-2 CD8+ T lymphocytes by lipid mediators in severe eosinophilic asthma. Mucosal Immunol. 2018 Sep;11(5):1408-1419.
- Chen W, et al. The Roles of Type 2 Cytotoxic T Cells in Inflammation, Tissue Remodeling, and Prostaglandin (PG) D2 Production Are Attenuated by PGD2 Receptor 2 Antagonism. J Immunol. 2021 Jun 1;206(11):2714-2724.
- Ye Y, et al. Neuromedin U promotes human type 2 immune responses. Mucosal Immunol. 2022 May;15(5):990-999.

3.4 Spatially mapping hepatic-immune interactions driving chronic liver pathology

Prof. Fadi Issa, Prof. Jane McKeating, Prof. Tao Dong & Dr. Amy Cross

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Project Overview

Spatially mapping hepatic-immune interactions driving chronic liver pathology

The liver carries out essential functions in metabolism, detoxification, protein synthesis, digestion, and in the storage of micronutrients. The constant flow of foreign food-derived and microbial antigens from the gut has necessitated adaptations in immune, stromal, and parenchymal compartments to avoid hypersensitivity or vulnerability to infectious disease. The hepatic niche has therefore taken on immunosuppressive features and mechanisms that not only support physiological function, but also can enable the acceptance of liver allografts, maintenance of chronic viral infections or even support tumour survival. The organ is made up of complex lobular and portal structures with distinct transcriptional and functional phenotypes created by gradients of oxygen exposure and metabolic characteristics. *We hypothesise that the distribution and cell-cell interactions of immune cell subsets within the hepatic architecture contributes to the processes of immunosurveillance and immunosuppression during chronic immune challenges.*

High plex technologies, such as single cell RNA sequencing, have described detailed transcriptional immune phenotypes, however the physical relationship of these immune cell subsets with stromal cells, parenchymal cells and histological features are not well known. Recent technological developments now enable us to characterise the gene expression of archived mammalian tissues by looking at 100s to 1000s transcripts in feature-driven or single cell-driven spatial analyses. Combining spatial transcriptomic approaches with multiplex imaging and proteomic approaches, we have an opportunity to dissect common and disease-specific immune cell-cell interactions that underpin tolerance and pathology in the human liver.

This project would firstly explore immune regulation in tissue biopsies from patients with chronic hepatitis B infection. HBV infection is a global health crisis, with over 290 million chronic infections worldwide resulting in over 800,000 deaths per year (WHO). A major challenge to curing this infection is the long-term persistence of viral cccDNA and the integration of the viral genome into the human genome within hepatocytes. Our recent data

has highlighted cellular heterogeneity in HBV infection status within each patient. In addition to achieving an in-depth characterisation of immune phenotypes, we aim to define the relationship between hepatocyte infection status, histological zonation, hepatic structures, and immune cells. We hypothesise that zonal hepatocyte-immune cell interactions regulate virus transcription. To investigate this, the candidate will participate in cutting-edge spatial transcriptomic experiments involving the Nanostring GeoMx or 10x Xenium platforms to collect high-plex RNA expression data. This will be accompanied by immunofluorescence imaging on the same tissue section or adjacent sections to determine cell identity, cell-cell interactions, and protein expression.

Liver tissue from healthy donors, hepatocellular carcinoma and liver transplant tolerance will be explored in comparative analysis to establish common and disparate immune cell interactions with and regulation by the parenchymal and stromal cells. The interpretation of cell-cell interactions would be supported by the mathematical modelling to challenge assumptions based on the static analysis of human tissue. The key transcriptional pathways and cellular interactions would be targeted in *in vitro* co-culture studies with cell lines and primary cells to establish their contribution to immunosuppression and activation.

The antigen-specificity of T lymphocytes is the context-dependent key to their activation driving cytotoxic, effector or even regulatory responses towards offending targets. The diversity and distribution of T cell clones will be measured by spatial transcriptomic methods within the different disease contexts. Furthermore, the candidate will develop protocols to assess T cell antigen specificity *in situ* using tetramer technology, which will be validated in humanised animal models and human tissue to provide a powerful tool in tissue biology analysis. The layers of transcriptomics, TCR specificity and proteomic data across chronic liver diseases will identify meaningful cell-cell and cell-structure interactions that give insight into pathogenesis, patient susceptibility and avenues for disease-specific intervention.

Disease Relevance

In 2017, the hepatitis B virus was associated with 1.4% of global deaths between acute hepatitis, liver cancer and cirrhosis secondary to the viral infection (GBD, 2017). The global prevalence of the virus is ~300 million people and it results in 0.7% of the total global disability-adjusted life-years.

Despite the use of antivirals and interferon therapy to manage acute viral replication, viral clearance is not achievable in most cases. The alternative functional cure is the seroconversion towards HBsAg negative, but many patients develop remission.

The scale and burden of hepatitis B is overwhelming. This project aims to understand at a high spatial resolution how chronic infection changes parenchymal, stromal and immune phenotype, distribution and interactions. We aim to understand cellular susceptibility and resistance to the virus in order to better understand the factors and kinetics that impact persistence and clearance.

Key Technology

Multiplex imaging – Immunofluorescence imaging of tissue covering up to 30 colours for identification and analysis of cell and histological features (Cell Dive). Use of fluorescent tetramers and T cell clones provided by the Tao lab to create protocols for *in situ* detection.

Transcriptomic spatial profiling – Gene expression of histological features or *in situ* single cells information within liver biopsies and explants and mapping this information back onto the diseased tissue (10X Xenium & NanoString GeoMx platforms).

Bioinformatics – Analysis of high-dimensional transcriptional datasets and multiplexed image analysis with the image of interpreting cell phenotypes, distributions, and interactions. Use of machine learning software (Visiopharm) and open-source R pipelines to explore data.

Training Opportunities

The candidate will receive practical training in tissue processing, basic histology, immunofluorescence, imaging, and multiple spatial transcriptomic platforms.

To enable data analysis, the student will be supported to undertake MSD training for to be able to use R/Python scripts and packages geared towards statistics, visualisations, and quantitative image analysis. Image analysis software currently used in the team includes machine-learning Visiopharm software, ImageJ and open-source QuPath interfaces. Cell interaction analyses will be carried out in collaboration with Oxford partners creating pipelines for network and neighbourhood analyses. Additional training will be sought out and provided based on needs and project progress.

Fundamental research skills the candidate will receive training in include experimental design, in vitro cell-based assays, data analysis, scientific writing and communication, and multidisciplinary research.

Day-to-day supervision and experimental training given by Dr Amy Cross. Regular meetings will be held for troubleshooting and experiment planning, in addition to monthly meeting with all supervisors to monitor the progresses. The project will be undertaken within the TRIG team at the IDRM on Old Road Campus (Headington) and the John Radcliffe Hospital (Headington) within the Nuffield Department of Surgical Sciences at the University of Oxford. The student would integrate into the team through attendance of weekly lab meetings, journal clubs and student catchups.

Key Publications

- Spatial transcriptomic characterization of COVID-19 pneumonitis identifies immune circuits related to tissue injury. A. R. Cross, C. E. de Andrea, M. Villalba-Esparza, M. F. Landecho, L. Cerundolo, P. Weeratunga, et al. JCI Insight 2023 Vol. 8 Issue 2
- Aggarwal, Abhishek, Pamela M. Odorizzi, Jens Brodbeck, Nicholas van Buuren, Christina Moon, Silvia Chang, Maryvic Adona, et al. 2023. “Intrahepatic Quantification of HBV Antigens in Chronic Hepatitis B Reveals Heterogeneity and Treatment-Mediated Reductions in HBV Core-Positive Cells.” *JHEP Reports : Innovation in Hepatology* 5 (4): 100664.
- Magri, Andrea, James M. Harris, Valentina D’Arienzo, Rosalba Minisini, Frank Jühling, Peter A. C. Wing, Rachele Rapetti, et al. 2022. “Inflammatory Gene Expression Associates with Hepatitis B Virus cccDNA- but Not Integrant-Derived Transcripts in HBeAg Negative Disease.” *Viruses* 14 (5). https://doi.org/10.3390/v14051070.
- Abd Hamid, Megat, Huw Colin-York, Nasullah Khalid-Alham, Molly Browne, Lucia Cerundolo, Ji-Li Chen, Xuan Yao, et al. 2020. “Self-Maintaining CD103+ Cancer-Specific T Cells Are Highly Energetic with Rapid Cytotoxic and Effector Responses.” *Cancer Immunology Research* 8 (2): 203–16.
- Montanari, Noe Rico, Ricardo Ramírez, Abhishek Aggarwal, Nick van Buuren, Michael Doukas, Christina Moon, Scott Turner, et al. 2022. “Multi-Parametric Analysis of Human Livers Reveals Variation in Intrahepatic Inflammation across Phases of Chronic Hepatitis B Infection.” *Journal of Hepatology* 77 (2): 332–43.

3.5 Understanding how super enhancers regulate gene expression

Prof. Doug Higgs & Dr Mira Kassouf

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Project Overview

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

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

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

enabling the full effect of the conventional enhancers.

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

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

Disease Relevance

Relevant to common forms of inherited of anaemia

Key Technology

Various techniques in molecular and computational biology

Training Opportunities

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

Key Publications

- Hay D, Hughes JR, Babbs C, Davies JOJ, Graham BJ, Hanssen L, Kassouf MT, Marieke Oudelaar AM, Sharpe JA, Suciu MC, Telenius J, Williams R, Rode C, Li PS, Pennacchio LA, Sloane-Stanley JA, Ayyub H, Butler S, Sauka-Spengler T, Gibbons RJ, Smith AJH,

Wood WG, **Higgs DR**. Genetic dissection of the α-globin super-enhancer in vivo. Nat Genet. 2016 Aug;48(8):895-903. doi: 10.1038/ng.3605. Epub 2016 Jul 4. PMID: 27376235; PMCID: PMC5058437.

- Larke MSC, Schwessinger R, Nojima T, Telenius J, Beagrie RA, Downes DJ, Oudelaar AM, Truch J, Graham B, Bender MA, Proudfoot NJ, **Higgs DR**, Hughes JR. Enhancers predominantly regulate gene expression during differentiation via transcription initiation. Mol Cell. 2021 Mar 4;81(5):983-997.e7. doi: 10.1016/j.molcel.2021.01.002. Epub 2021 Feb 3. PMID: 33539786.
- Hua P, Badat M, Hanssen LLP, Hentges LD, Crump N, Downes DJ, Jeziorska DM, Oudelaar AM, Schwessinger R, Taylor S, Milne TA, Hughes JR, **Higgs DR**, Davies JOJ. Defining genome architecture at base-pair resolution. Nature. 2021 Jul;595(7865):125-129. doi: 10.1038/s41586-021-03639-4. Epub 2021 Jun 9. PMID: 34108683.
- Oudelaar AM, Higgs DR. The relationship between genome structure and function. Nat Rev Genet. 2021 Mar;22(3):154-168. doi: 10.1038/s41576-020-00303-x. Epub 2020 Nov 24. PMID: 33235358.
- Joseph Blayney, Helena Francis, Brendan Camellato, Leslie Mitchell, Rosa Stolper, Jef Boeke, **Douglas Higgs***, Mira Kassouf* Super-enhancers require a combination of classical enhancers and novel facilitator elements to drive high levels of gene expression. bioRxiv 2022.06.20.496856; doi: https://doi.org/10.1101/2022.06.20.496856 (2023 Cell in press).

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

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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

Relevant to common forms of inherited of anaemia.

Key Technology

Various techniques in molecular and computational biology

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

- Buckley M, Hua P, Suciu MC, Marieke Oudelaar A, Hanssen LLP, Jeziorska D, Roberts N, Carpenter SJ, Francis H, Telenius J, Olijnik AA, Sharpe JA, Sloane-Stanley J, Eglinton

J, Kassouf MT, Orkin SH, Pennacchio LA, Davies JOJ, Hughes JR, **Higgs DR**, Babbs C. Reactivation of a developmentally silenced embryonic globin gene. Nat Commun. 2021 Jul 21;12(1):4439. doi: 10.1038/s41467-021-24402-3. PMID: 34290235; PMCID: PMC8295333.

- Mettananda S, Fisher CA, Hay D, Badat M, Quek L, Clark K, Hublitz P, Downes D, Kerry J, Gosden M, Telenius J, Sloane-Stanley JA, Faustino P, Coelho A, Doondeea J, Usukhbayar B, Sopp P, Sharpe JA, Hughes JR, Vyas P, Gibbons RJ, **Higgs DR**. Editing an α-globin enhancer in primary human hematopoietic stem cells as a treatment for β-thalassemia. Nat Commun. 2017 Sep 4;8(1):424. doi: 10.1038/s41467-017-00479-7. PMID: 28871148; PMCID: PMC5583283.
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- Oudelaar AM, Beagrie RA, Kassouf MT, Higgs DR. The mouse alpha-globin cluster: a paradigm for studying genome regulation and organization. Curr Opin Genet Dev. 2021 Apr;67:18-24. doi: 10.1016/j.gde.2020.10.003. Epub 2020 Nov 19. PMID: 33221670; PMCID: PMC8100094.
- Songdej D, Babbs C, Higgs DR; BHFS International Consortium. An international registry of survivors with Hb Bart's hydrops fetalis syndrome. Blood. 2017 Mar 9;129(10):1251-1259. doi: 10.1182/blood-2016-08-6

3.7 Role of ubiquitin specific protease USP19 in adipogenesis and immune signalling by altering steroid hormone metabolism

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Project Overview

Ubiquitin specific protease 19 (USP19) is a membrane bound deubiquitinase involved in eliminating misfolded proteins and cellular debris and regulating NFκB signalling. Recent studies have implicated USP19 in glucocorticoid and insulin signalling. USP19-deficient mice exert a lean phenotype, are less prone to obesity and insulin resistance and exert higher levels of testosterone. Adipose and muscle tissues extracted from USP19 knockout mice were analysed using advanced proteomics and ubiquitomics pipelines, revealing altered pathways involved in adipogenesis, thermogenesis, and insulin and glucocorticoid signalling.

The aim of this project is to study the molecular mechanism of USP19 in these pathways by identifying and characterising the direct deubiquitination targets of USP19. Also, we shall explore the role of USP19 in innate immune signalling, glucocorticoid signalling and steroid metabolism.

Disease Relevance

The identification of USP19-dependent critical enzymes involved in cholesterol metabolism with potential subsequent effects on glucocorticoid and steroid/testosterone levels may point towards the therapeutic potential of USP19 inhibition in obesity-related loss of lean muscle mass (OLLMM), sarcopenia and autoimmune disorders.

Key Technology

Proximity labelling, advanced mass spectrometry for proteomics and lipidomics

Training Opportunities

This project will provide training opportunities in laboratory techniques such as tissue culture, protein expression and deletion in cell models, proximity labelling and analysis by mass spectrometry for proteomics and lipidomics studies.

Key Publications

- Olie CS, Pinto-Fernández A, Damianou A, Vendrell I, Mei H, den Hamer B, van der Wal E, de Greef JC, Raz V, Kessler BM. USP18 is an essential regulator of muscle cell differentiation and maturation. ***Cell Death Dis.*** 2023 Mar 31;14(3):231. doi: 10.1038/s41419-023-05725-z.
- Dietz L, Ellison CJ, Riechmann C, Cassidy CK, Felfoldi FD, Pinto-Fernández A, Kessler BM, Elliott PR. Structural basis for SMAC-mediated antagonism of caspase inhibition by the giant ubiquitin ligase BIRC6. ***Science***. 2023 Mar 17;379(6637):1112-1117. doi: 10.1126/science.ade8840.
- Pinto-Fernandez A, Salio M, Partridge T, Chen J, Vere G, Greenwood H, Olie CS, Damianou A, Scott HC, Pegg HJ, Chiarenza A, Díaz-Saez L, Smith P, Gonzalez-Lopez C, Patel B, Anderton E, Jones N, Hammonds TR, Huber K, Muschel R, Borrow P, Cerundolo V, Kessler BM. Deletion of the deISGylating enzyme USP18 enhances tumour cell antigenicity and radiosensitivity. ***Br J Cancer***. 2021 Feb;124(4):817-830. doi: 10.1038/s41416-020-01167-y.
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- Turnbull AP, Ioannidis S, Krajewski WW, Pinto-Fernandez A, Heride C, Martin ACL, Tonkin LM, Townsend EC, Buker SM, Lancia DR, Caravella JA, Toms AV, Charlton TM, Lahdenranta J, Wilker E, Follows BC, Evans NJ, Stead L, Alli C, Zarayskiy VV, Talbot AC, Buckmelter AJ, Wang M, McKinnon CL, Saab F, McGouran JF, Century H, Gersch M, Pittman MS, Marshall CG, Raynham TM, Simcox M, Stewart LMD, McLoughlin SB, Escobedo JA, Bair KW, Dinsmore CJ, Hammonds TR, Kim S, Urbé S, Clague MJ, Kessler BM, Komander D. Molecular basis of USP7 inhibition by selective small-molecule inhibitors. ***Nature.*** 2017 Oct 26;550(7677):481-486. doi: 10.1038/nature24451.

3.8 Deciphering the composition, structure and function of extracellular particles in the pathogenesis of pleural effusions

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Project Overview

Clinical Background: The lungs are lined by a double layer of mesothelial cells. The area in-between these two membranes is called the pleural cavity. Established Pleural Infection (EPI) is a severe and complicated disease where bacteria invade the pleural space, establish a niche and cause a pleural effusion (accumulation of fluid in the pleural cavity). EPI bears a considerable morbidity and mortality worldwide. Epidemiological data shows increasing incidence, hospitalizing over 80,000 individuals in the United Kingdom (UK) and United States (US) each year.

The clinical problem: The clinical outcomes of EPI remain poor. Patients need prolonged hospitalization (mean: 14 days) and often require invasive treatments such as surgery. Recent studies demonstrate mortality of 20%, rising to 40% in vulnerable populations such as the elderly. The exact pathways of pleural effusion pathogenesis and progression remain unknown with potential involvement of infections and malignancies.

Recent data indicates that most infection related EPIs are polymicrobial[1] with increased Plasminogen Activator Inhibitor 1 (PAI-1[2]) levels and intrapleural fibrinolytic activity. This results in accumulation of fibrin which is causing pleural septations and the segmentation of the pleural space. EPI patients require total drainage of the pleural effusion to treat the disease, which is hardened by the presence of excess fibrin.

Evidence shows that extracellular vesicles (EVs) are involved in idiopathic pulmonary fibrosis and are key mediators of fibrinolysis in the context of cancer[3]. However, little is known regarding the role of extracellular particles and EVs from bacterial, immune or tumor origin in the fibrinolytic activity of pleural effusions.

Research Opportunities and Impact: Extracellular vesicles (EVs) are essential trans-cellular messengers released by a number of cells including immune, stromal, tumor and bacterial. Some evidence suggests that EVs regulate immunity and tolerance by serving as vehicles for a number of signals exchanged between stromal and immune cells. Among others, EVs rely immune receptors, regulatory cytokines, pro-inflammatory lipids and genetic

material, including small non-coding RNAs and messenger RNAs to recipient cells[4,5]. The biochemical diversity, structure and role of pleural extracellular particles is not known, especially in pathological conditions such as cancer and infections, and despite both tumor and bacteria produce EVs, little is known regarding their role in the pathogenesis and progression of malignant pleural effusions. In an attempt to identify molecular drivers of disease, we plan to perform the first multi-OMIC and functional characterisation of pleural extracellular particles. We will also profile plasma-associated extracellular particles to identify circulating EVs indicative of pleural compromise and prognosis.

Methods and Outlook: We will collect paired pleural fluid and blood specimens from patients with EPI. These samples will be subjected to a variety of methods to ensure proper separation of cells from plasma, and pleural fluid. These different fractions will be profiled using a multifaceted approach harnessing cutting-edge technologies at the CAMS Oxford Institute and the Old Road Campus. For instance, isolated T cells and cancer cells will be profiled by high-dimensional flow cytometry, scRNAseq, bulk proteomics, bulk PTM-proteomics (including glycomics) and a variety of functional assays[6,7]. We will test if and how different acellular fractions within pleural effusions, rich in either EVs, proteinaceous particles and cytokines interfere with T-cell activation, synapse assembly and overall T-cell communication[8,9]. Untargeted OMIC analyses will be performed to identify critical regulators of T-cell dysfunction in the context of cancer and infections.

Disease Relevance

Our goal is to perform a comprehensive profiling of extracellular particles from pleural effusion specimens derived from pleural infection patients to identify molecular determinants of disease and predictors of clinical outcomes. We will analyse how pleural effusion (*in situ*) and either bacterial- or mesothelioma-derived (*in vitro*) extracellular particles modulate inflammation and the scalation of adaptive immune responses. This could lead to the discovery of novel disease progression and therapeutic biomarkers.

Key Technology

Super-resolution microscopy, Airyscan microscopy, high-dimensional flow cytometry, nano flow cytometry, proteomics, transcriptomics and data analysis.

Training Opportunities

In all abovementioned methods.

Key Publications

- Kanellakis, N. I. *et al.* The bacteriology of pleural infection (TORPIDS): an exploratory metagenomics analysis through next generation sequencing. *Lancet Microbe* **3**, e294-e302 (2022). https://doi.org:10.1016/S2666-5247(21)00327-X
- Bedawi, E. O. *et al.* The Biological Role of Pleural Fluid PAI-1 and Sonographic Septations in Pleural Infection: Analysis of a Prospectively Collected Clinical Outcome Study. *Am J Respir Crit Care Med* **207**, 731-739 (2023). https://doi.org:10.1164/rccm.202206-1084OC
- Durrieu, L., Bharadwaj, A. & Waisman, D. M. Analysis of the thrombotic and fibrinolytic activities of tumor cell-derived extracellular vesicles. *Blood Adv* **2**, 1054-1065 (2018). https://doi.org:10.1182/bloodadvances.2017015479
- H, U. S., Brotherton, D. & Inal, J. Communication is key: extracellular vesicles as mediators of infection and defence during host-microbe interactions in animals and plants. *FEMS Microbiol Rev* **46** (2022). https://doi.org:10.1093/femsre/fuab044
- Berumen Sanchez, G., Bunn, K. E., Pua, H. H. & Rafat, M. Extracellular vesicles: mediators of intercellular communication in tissue injury and disease. *Cell Commun Signal* **19**, 104 (2021). https://doi.org:10.1186/s12964-021-00787-y

3.9 Thymic involution & T Cell Aging

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Project Overview

A significant aspect of aging is the decline in immune response. One of the most recognized consequences of immune system aging is thymic involution. This project focus on investigating the impact of thymic involution on CD8 T aging and dysfunction. Specifically, we will investigate whether thymic involution affects T cell differentiation, function, and proliferation using mouse models and cutting edging molecular biology techniques.

Disease Relevance

Aging induced T cells dysfunction reduces anti-infection and anti-tumor immune response, as well as vaccination. It is also associated with a higher prevalence of autoimmunity.

Key Technology

Multiparameter flow cytometry; Single cell RNA sequencing; Adoptive cell transfer; Primary T cell culture; Mice breeding and genotyping

Training Opportunities

Students will be trained in a range of laboratory techniques including mouse experiments and primary cell culture as well as basic molecular and cell biology techniques. Students will also have the opportunities to present his/her work at lab meetings, institute internal seminars and domestic and international conferences.

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