2021 PhD projects and supervisory teams
Doctoral Fellowships for Clinicians

THE FRANCIS CRICK INSTITUTE

The role of "persistent PAMPs" in the cytokine storm and ARDS during severe COVID-19 and highly pathogenic influenza infection. Supervisory team: David Bauer (primary supervisor, Crick) and Mahdad Noursadeghi (UCL).

Inflammation associated with SARS-CoV-2 infection. Supervisory team: Rupert Beale (primary supervisor, Crick) and Wendy Barclay (Imperial College London)

Effect of acute myeloid leukaemia on normal haematopoiesis. Supervisory team: Dominique Bonnet (primary supervisor, Crick) and David Taussig (The Institute of Cancer Research, Royal Marsden)

Investigation and modelling of multiple myeloma pathogenetic evolution. Supervisory team: Dinis Calado (primary supervisor, Crick), Richard Houlston (The Institute of Cancer Research) and Martin Kaiser (The Institute of Cancer Research, Royal Marsden)

TGF-β family ligands as potential therapeutic targets in pancreatic cancer. Supervisory team: Caroline Hill (primary supervisor, Crick) and Debashis Sarker (King's College London)

Development of multisynaptic tracing technologies to target and manipulate disease-relevant neural circuits. Supervisory team: Johannes Kohl (primary supervisor, Crick), Andrew Murray (UCL) and Rickie Patani (Crick/UCL)

Human Cell Atlas: single-cell transcriptomics of vitiligo. Supervisory team: Nicholas Luscombe (primary supervisor, Crick), Magnus Lynch (King's College London) and John Ferguson (King's College London)

Structural and biochemical analysis of Salmonella effector functions and their interaction with host proteins. Supervisory team: Katrin Rittinger (primary supervisor, Crick), Teresa Thurston (Imperial College London) and Rupert Beale (Crick)

Developing new viral vectors for *in vivo* gene therapy. Supervisory team: Sam Rodriques (primary supervisor, Crick), Matthew Walker (UCL) and Rob Brownstone (UCL)

Identifying and functionally characterising non-coding drivers of renal cancer evolution. Supervisory team: Samra Turajlic (primary supervisor, Crick) and Richard Houlston (The Institute of Cancer Research)

Evaluating amino acid-based PET tracers to predict metabolic sensitivities in breast cancers. Supervisory team: Mariia Yuneva (primary supervisor, Crick), Ashley Groves (UCL), Stefan Voo (UCL) and Neill Patani (UCL)

The role of “persistent PAMPs” in the cytokine storm and ARDS during severe COVID-19 and highly pathogenic influenza infection

A PhD project for the 2021 doctoral clinical fellows programme with David LV Bauer (primary supervisor, Crick) and Mahdad Noursadeghi (UCL)

The activation of innate immune responses following infection is driven by the detection of pathogen-associated molecular patterns (PAMPs). For RNA viruses, the structured viral genomic RNA itself constitutes the main PAMP, and detection by host receptors (TLR3, RIG-I, or MDA5) leads to interferon production via MAVS signalling of NF-κB and IRF1/3, and the subsequent generation of an antiviral state and recruitment of lymphocytes to the site of infection.

While some viruses have evolved to cope with interferon activation (e.g. paramyxoviruses), diverse others have evolved to avoid detection in the first place by “hiding” their PAMPs. Both SARS-CoV-2 (a positive-sense RNA virus) and Influenza A virus (IAV, a negative-sense RNA virus) have adopted this strategy: SARS-CoV-2 encodes a nuclease to digest double-stranded RNA, and IAV encodes a viral polymerase that tightly binds its complementary RNA termini. Consequently, under ‘normal’ conditions, interferon activation is suppressed during SARS-CoV-2 (Blanco-Melo et al., 2020) and influenza (te Velthuis, Long, Bauer, et al., 2018) infection. Cell death, however, results in a release of a significant quantity of unmasked, stable, structured viral RNA that could serve as PAMPs to drive continued inflammation, even following viral clearance.

This project aims to understand the role of structured viral RNA in driving immune activation and inflammation during infection with SARS-CoV-2 and IAV. We will take two approaches: first, characterising the PAMPs that persist following viral clearance in patient samples, and second, using cell culture and animal models of infection to determine the mechanism by which structured RNAs drive inflammation, cytokine induction, and the development of ARDS.

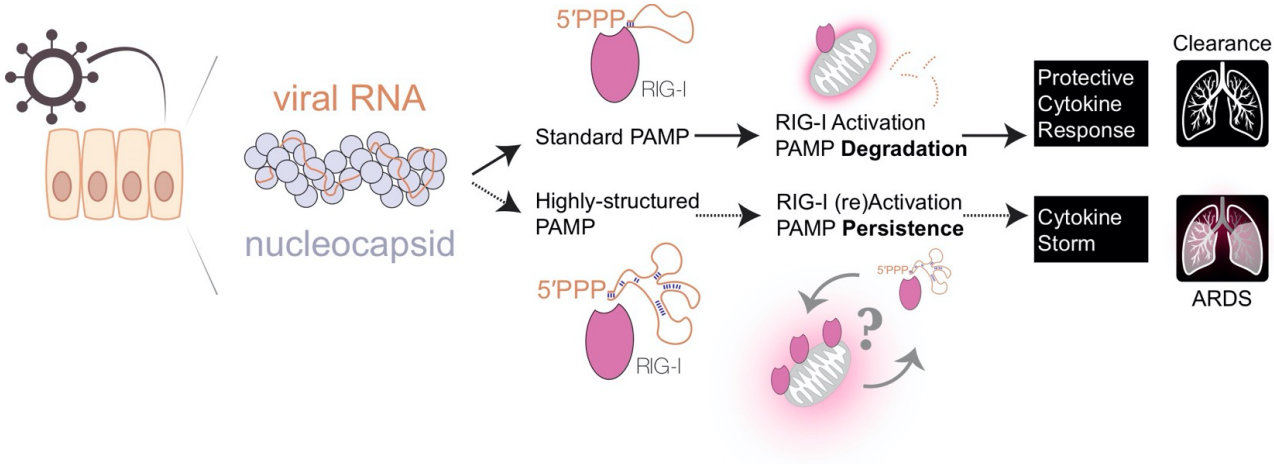
This work builds on our recent successes in mapping the viral RNA structure of SARS-CoV-2 (Knight et al., 2020) and influenza A virus (Dadonaite et al., 2020) — as well as our work on understanding the RNA structures that lead to interferon activation during H5N1 and 1918 pandemic influenza virus infection (te Velthuis, Long, Bauer, et al., 2018).

For the first aspect of the project, we will use next-generation sequencing to identify the specific viral RNA fragments that persist in patient samples, and use single-molecule RNA structure probing methods to identify the structural motifs that they adopt. In combination with our ongoing work on viral RNA structure, we expect to develop a better understanding of which regions of the viral genome are immunogenic.

In the second aspect of the project, we will examine the cellular pathways that are activated in response to persistent viral RNA, and the features of RNA structure that contribute to persistence within specific cell types (e.g. dendritic cells). Using animal models of COVID-19 infection, we will isolate single cells within infected tissue and use high-throughput analyses (sequencing, proteomics, flow cytometry) to examine the function of persistent PAMPs in specific cell states and types.

Critically, these results will inform the development of diagnostic assays (e.g. explaining the long duration that COVID-19 patients test positive for viral RNA by RT-qPCR, despite not shedding any live virus — Wölfel et al., 2020), and potential therapeutics (e.g. targeted degradation via CRISPR/Cas9 to clear persistent PAMPs).

The partner institution for this project is UCL.



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Inflammation associated with SARS-CoV-2 infection

A PhD project for the 2021 doctoral clinical fellows programme with Rupert Beale (primary supervisor, Crick) and Wendy Barclay (Imperial College London)

The striking effectiveness of dexamethasone in treating ventilated Covid-19 patients suggests that most severe pathology caused by SARS-CoV-2 may be due to inflammation. At a molecular level this may be caused in part by the SARS-CoV-2 envelope (E) protein. E is thought to form an ion channel, a so called 'viroporin'. It is likely that this plays a role in ensuring that the main entry weapon of the virus – spike glycoprotein – maintains the correct conformation during assembly of the virus. Influenza A virus encodes a viroporin (M2) that plays a similar role, preventing the premature triggering of Haemaglutinin. Both viroporins trigger similar pathways, suggesting that perturbations of intracellular ion gradients by viruses may be highly inflammatory. Notably, M2 is the target of antiviral drugs. Small molecule inhibitors of E under development may therefore be effective both as antivirals and anti-inflammatory agents.

This project will build on ongoing characterization of the pathways involved to explore the roles of E and M2 in generating inflammation in various model systems. These may comprise in vitro or in vivo models, and in general will be whatever emerging systems represent the best and most tractable approximation of human disease.

The partner institution for this project is Imperial College London.

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Effect of acute myeloid leukaemia on normal haematopoiesis

A PhD project for the 2021 doctoral clinical fellows programme with Dominique Bonnet (primary supervisor, Crick) and David Taussig (The Institute of Cancer Research, Royal Marsden)

Acute myeloid leukaemia (AML) disrupts the generation of normal blood cells, predisposing patients to haemorrhage, anaemia, and infections. Differentiation and proliferation of residual normal hematopoietic stem and progenitor cells (HSPCs) are impeded in AML-infiltrated bone marrow (BM). The underlying mechanisms and interactions of residual hematopoietic stem cells (HSCs) within the leukemic niche are poorly understood, especially in the human context.

Our previous work on AML patient samples and patient-derived xenograft (PDX) models demonstrated an enrichment of HSCs at the expense of less-primitive progenitors among the remaining normal hematopoietic cells in the BM (1). Consistent with this, most younger patients achieve rapid reversal of marrow failure on attainment of remission following intensive chemotherapy. AML-induced cytopenias might be expected to drive residual HSPCs into cell cycle through feedback, but HSPCs are predominantly quiescent (1-3) and express aberrant levels of the negative cell cycle regulators *p19*, *p21*, and *Egr1-3* (4). These observations imply that BM failure is not a consequence of HSC depletion but rather involve dysregulation of cell cycle activation and differentiation. This present project will make used of a humanized model to study the crosstalk among HSPCs, leukemia, and their MSC niche, and a molecular mechanism whereby AML impairs normal haematopoiesis. Indeed, to mimic AML infiltration and dissect the cellular crosstalk in human BM, we established humanized ex vivo and in vivo niche models comprising AML cells, normal HSPCs, and mesenchymal stromal cells (MSCs). Both models replicated the suppression of phenotypically defined HSPC differentiation without affecting their viability.

Using these two models, we recently reported that HSPC suppression is largely dependent on secreted factors produced by transcriptionally remodeled MSCs. One of this factor is MSC-derived stanniocalcin 1 (STC1) and its transcriptional regulator HIF-1α (5). Nevertheless, how STC1 is affecting normal HSCs and why AML cells are refractory to the effect of STC1 is still unknown. The project will thus focus on dissecting how SCT.1 affect normal HSC, whether STC1 level in patients' samples could be a predictor of clinical outcome but also investigate other potential secreted factors that contribute to the BM failure.

The partner institution for this project is The Institute of Cancer Research.

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Investigation and modelling of multiple myeloma pathogenetic evolution

A PhD project for the 2021 doctoral clinical fellows programme with Dinis Calado (primary supervisor, Crick), Richard Houlston (The Institute of Cancer Research) and Martin Kaiser (The Institute of Cancer Research, Royal Marsden)

Multiple myeloma (MM) is an incurable disease of antibody secreting cells (plasma cells, PCs) for which biological understanding is poor and an unmet need exists to develop effective therapies. During the course of a T-cell dependent immune response to infection B-cells (e.g. in spleen and lymph nodes) differentiate into PCs in a reaction called the germinal center (GC). After generation, PCs home to specialised microenvironments in the bone marrow that are critical for their survival. The pathogenesis of MM is complex and poorly understood. Currently it is considered that the initiating oncogenic mutation occurs in a GC B-cell, e.g. a chromosomal translocation that leads to the overexpression of an oncogene (CCND1, CCND3, MAF, MAFB, WHSC1/MMSET (alias NSD2), and FGFR3). Additional mutations (e.g. KRAS, NF-κB, P53 and MYC) are then acquired after B-cell differentiation in PCs in the bone marrow[1]. These latter mutations are thought to overcome microenvironment dependencies leading to increased disease aggressiveness and failure of patients to respond to current therapies.

Grounded on the expertise in GC B-cell and PC biology (Calado)[2, 3], MM pathogenesis, genetics and therapies (Houlston and Kaiser)[4, 5], this project aims to deepen the current understanding of the pathogenetic evolution of MM, generate bona-fide clinically relevant mouse models of disease, and identify novel therapeutic strategies.

(PhD Year 1-2): Modelling the modular acquisition of oncogenic mutations i.e. first in a GC B-cell and after that in a PC is a major technical challenge, hindering the generation of mouse models faithfully reproducing MM pathogenesis and evolution. Whereas the introduction of oncogenic mutations in GC B-cells is common practice[2], the same is not true for PCs. However, in a major breakthrough we have developed a system in the mouse that allows for the first time to introduce oncogenic mutations specifically in PCs (Calado)[3]. The development of MM mouse models will be guided by current and to be gathered knowledge on the pathogenetic evolution of human MM (Houlston). The MM mouse models will allow the investigation of disease progression and alterations on the cellular populations in the tumour microenvironment, including their functional state.

(PhD Year 2-3): These studies will run in parallel with trial-specific myeloma biobanking from multi-centric UK NCRI phase III trials and phase I/II studies in the Myeloma UK Clinical Trials Network (Kaiser)[5]. This provides an opportunity for inter-species (human/mouse) MM pathological, oncogenomic and gene expression comparisons to identify alterations that function as biomarkers of therapy success and/or that allow to risk-stratify patients to the appropriate treatment. Using that information we will perform the functional testing of the impact of candidate oncogenic mutations on the outcome of the various treatments in the MM mouse models.

The project training skills include high throughput DNA/RNAsequencing; single cell analysis using RNAsequencing, multi-colour flow-cytometry and CyTOF; bioinformatics of high content data; pathological characterisations of affected mouse and human tissues using immunohistochemistry, fluorescence in-situ hybridization, image mass-cytometry; whole mouse body imaging techniques including PET and ultrasounds; genetic manipulation using CRISPR-CAS9 technology; and advanced knowledge of mouse models of disease.

The partner institution for this project is The Institute of Cancer Research.

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TGF-β family ligands as potential therapeutic targets in pancreatic cancer

A PhD project for the 2021 doctoral clinical fellows programme with Caroline Hill (primary supervisor, Crick) and Debashis Sarker (King's College London)

The Transforming Growth Factor β (TGF-β) signalling pathways have long been known to play important roles in cancer and can act both as tumour suppressors and tumour promoters, depending on the stage of tumourigenesis [1, 2]. These pathways are transduced through serine/threonine kinase receptors and the SMAD proteins, which upon being phosphorylated in response to ligand stimulation, act as transcription factors in the nucleus to regulate the transcription of target genes [1]. Late-stage tumours of many different tissue origins exhibit enhanced phosphorylated SMAD2/3 staining and transcriptional signatures that have been attributed to TGF-β activity [3,4]. However, recent work in the Hill lab at the Crick has revealed that long-term exposure to TGF-β paradoxically results in low level signaling [5]. Thus, we hypothesized that in *in vivo* pathological contexts, such as primary tumours or metastases, where high levels of phosphorylated SMAD2/3 have been observed, the tumour-promoting ligand responsible may not actually be TGF-β itself, but other TGF-β family ligands, that share the same downstream pathway.

We have now identified two closely-related TGF-β family ligands that are secreted by cancer-associated fibroblasts (CAFs) and are responsible for high levels of phosphorylated SMAD2/3 in a mouse model of pancreatic ductal adenocarcinoma (PDAC), one of the hardest to treat cancers. We have shown that secretion of these ligands leads to exclusion of immune cells in the tumours. We have developed a human antibody that is capable of neutralizing these ligands in mouse and humans and have exciting preliminary evidence that ligand neutralization leads to higher infiltration of T cells in tumours in a mouse model of PDAC. This suggests that combining this novel antibody with immune checkpoint inhibitors could be a promising approach for treating PDAC patients.

The proposed project has three main aims:

Firstly, the student will generate a 'high ligand signature' by performing RNA-seq on normal fibroblasts treated ± the relevant TGF-β family ligands, and also on CAFs from cancer patients, which we predict to express high levels of these ligands treated *in vitro* ± neutralizing antibody. We propose to use this signature to analyse tumours from patients treated with immune checkpoint inhibitors, to determine whether it is capable of predicting which patients might respond to combination therapy comprising immune checkpoint inhibitors and the neutralizing antibody.

Secondly, the student will use orthotopic mouse models of PDAC to test the efficacy of combining immune checkpoint inhibitors with our neutralizing antibody in reducing tumour growth and spread. The student will address by immunophenotyping whether the neutralizing antibody reduces immune suppression and thus sensitizes the tumours to immune checkpoint inhibitors.

Finally, our preliminary data has indicated that PDAC patients have elevated levels of ligands in their circulation compared with age-matched normals and also, we have detected high levels of these ligands in PDAC tumours. The student will collect human PDAC samples and matched longitudinal bloods to understand how levels of ligand in tumours correlate with disease stage and different treatments, and also determine whether ligand levels in tumours correlate with circulating levels of ligand.

The partner institution for this project is King's College London.

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Development of multisynaptic tracing technologies to target and manipulate disease-relevant neural circuits

A PhD project for the 2021 doctoral clinical fellows programme with Johannes Kohl (primary supervisor, Crick), Andrew Murray (UCL) and Rickie Patani (Crick/UCL)

There is increasing evidence that network dysfunction is a key aspect of many neurological disorders [1], but our ability to therapeutically access specific neural circuits in the central nervous system (CNS) is quite limited. Viral vectors such as adeno-associated viruses (AAVs) have been successfully used for gene therapy and have considerable potential for gene delivery to the CNS [2]. However, AAVs do not cross synapses and cannot be targeted to the precise subcircuits that underlie many nervous system disorders. There is thus a need for therapeutic agents that target specific brain circuits.

In recent years, several modifications have rendered trans-synaptic rabies virus (RABV) into a promising tool for this purpose. Genetic engineering has resulted in RABV variants with very low or absent toxicity [3,4] and deletion mutants were introduced that are capable of identifying and targeting monosynaptically coupled neurons [5]. Despite this progress, we lack tools to visualize and interrogate multisynaptically connected neural circuits. Development of such tools could form the basis of a new generation of gene therapy vectors.

The goal of this project will thus be to engineer a multisynaptic neural tracing system to delineate CNS circuits and to deliver gene editing agents (e.g. CRISPR/Cas9) to specific components of these circuits. The candidate will design and produce custom viral vectors (AAVs, RABV) and test them in mouse models by performing anatomical and functional studies in clinically relevant circuits (e.g. dopaminergic circuits, involved in addictive behaviour). This project will build on existing work in the Kohl group (Crick) and will be co-supervised by Dr Andy Murray (Sainsbury Wellcome Centre, UCL), an expert in spinal circuits and viral tracing. In addition, Prof. Rickie Patani (Crick, UCL), a clinician and molecular neuroscientist, will provide clinical expertise and career guidance. The successful candidate will be part of a vibrant neuroscience community and will have access to state-of-the-art facilities at the host institute(s).

This will be an exciting opportunity to use systems and cellular neuroscience approaches – such as *in vivo* miniature microscopy, behavioural profiling, viral tracing and (spatial) transcriptomics – to both contribute towards the development of network-targeted therapy for circuit dysfunctions and to address key questions in neuroscience. Please contact Dr Kohl to discuss further details.

Candidate background: Candidates should have a keen interest in neuroscience. We are looking for highly motivated and creative individuals that thrive in a collaborative setting. Since this project will combine aspects from cellular and circuit neuroscience, an openness to learn new techniques and approaches is desirable.

The partner institution for this project is UCL.

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Human Cell Atlas: single-cell transcriptomics of vitiligo

A PhD project for the 2021 doctoral clinical fellows programme with Nicholas Luscombe (primary supervisor, Crick), Magnus Lynch (King's College London) and John Ferguson (King's College London)

The role of metabolic reprogramming in different aspects of tumorigenesis is now well established. Melanocytes are neural-crest derived cells that are primarily responsible for the production of pigment in vertebrates. In humans they are located at the basal layer of the epidermis and provide melanin pigment for skin, mucosa, hair and the uveal surface in the eye. Melanocytes additionally have antigen presentation functions and play a role in cell-mediated immunity. Vitiligo is an autoimmune disease characterized by CD8+ T cell-mediated attack on melanocytes leading to macular patches of depigmentation. This has severe psychosocial consequences and current therapeutic approaches are frequently ineffective. The impact of Vitiligo is particularly severe in BAME (Black Asian and Minority Ethnic) groups and has been undertreated in the past by dermatologists. Vitiligo can broadly be classified into non-segmental vitiligo, which affects multiple sites on the body symmetrically and segmental vitiligo. Segmental vitiligo usually involves unilateral band like involvement at a single location. Non-segmental vitiligo can exhibit generalised, acrofacial, or universalis patterns. We hypothesise that segmental vitiligo reflects a somatic mosaicism of melanocytes and/or keratinocytes within affected sites. This leads to the formation of a neoantigen that predisposes to autoimmune attack. Understanding the nature of this neoantigen may give insight into the immunological mechanisms of all types of vitiligo. At present, the gold standard therapy for vitiligo remains "narrow-band" UVB phototherapy. The mechanism of action of this treatment remains poorly understood. Understanding it may lead to the development of new and more effective treatments for vitiligo.

The aims of the project are as follows:

1. To explore melanocyte heterogeneity in normal skin through the bioinformatics analysis of existing single cell transcriptomics data.
2. To undertake single cell transcriptomic analysis of skin from affected and unaffected areas in patients with segmental and generalized vitiligo.
3. To undertake cell transcriptomic analysis of skin from affected areas in patients before and after successful treatment with phototherapy.
4. To assist in the design (including ethical application) of a trial of autologous melanocyte transplantation in the treatment of segmental vitiligo.
5. To undertake exome and/or whole genome sequencing of DNA from melanocytes derived from affected areas of skin in segmental vitiligo and to compare to unaffected skin and blood to understand whether somatic mosaicism is present.

The partner institution for this project is King's College London.

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Structural and biochemical analysis of Salmonella effector functions and their interaction with host proteins

A PhD project for the 2021 doctoral clinical fellows programme with Katrin Rittinger (primary supervisor, Crick), Teresa Thurston (Imperial College London) and Rupert Beale (Crick)

Many bacterial pathogens deliver virulence (effector) proteins into mammalian host cells that enable bacterial growth and disease progression (1). This is often achieved by high-jacking and subverting host cellular processes. In collaboration with the group of Teresa Thurston at Imperial College, Department of Infectious Diseases, we have characterised several Salmonella effectors that interfere with innate immune responses. For example, SseK1 and SseK3 inhibit NF-κB signalling and inflammatory necroptotic cell death and we found SseK3 to be a retaining N-acetyl glucosamine transferase (2). In contrast, GtgA family members are proteases that cleave specific NF-κB subunits (3).

These and other studies have revealed that the innate immune response has been a major selective pressure for effectors that suppress its activation. Of the 28 known effectors delivered into host cells via the SPI2-encoded type III secretion system, several, including GogB (reported to inhibit NF-κB), SifB (unknown function) and SteE (STAT3 activation) (4) remain to be structurally and mechanistically characterized. Furthermore, there are novel effectors for which very little is known.

This project will use structural biology approaches combined with biochemical methods and cell biology to determine, at the molecular level, how an effector functions and targets host proteins, which has the potential to highlight novel strategies to target bacterial pathogens.

Effector candidates will be selected, and biochemical techniques will be used to establish effector-host protein-protein interactions (pull-downs, co-immune precipitation, mass spectrometry) with infection-based assays used to validate candidates from these or published studies. Upon identifying bone fide host interactors, we will apply structural methods (X-ray crystallography and NMR spectroscopy) to characterize the effector protein and its interaction with host factors (see for example (5)). Biochemical analysis will be performed to investigate putative post-translational modifications and their significance. The physiological significance of the effector-host protein interaction will be explored using site-directed mutagenesis to abrogate the binding interface, CrispR-cas9 mutagenesis of host cells to determine the functional significance of the effector-host protein interaction and confocal cell immunofluorescence to establish protein localization and effector delivery at the single cell level.

The majority of the project including structural and biochemical assays, and mass spectrometry will be conducted in the Rittinger lab at the Francis Crick Institute. Infection work will be carried out in the Thurston lab in the MRC Centre for Molecular Bacteriology and Infection, at the South Kensington Campus of Imperial College London using techniques that are well established in the Thurston group.

The partner institution for this project is Imperial College London.

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Developing new viral vectors for *in vivo* gene therapy

A PhD project for the 2021 doctoral clinical fellows programme with Sam Rodriques (primary supervisor, Crick), Matthew Walker (UCL) and Rob Brownstone (UCL)

The Applied Biotechnology Laboratory at the Crick Institute develops first-in-class technologies that can be deployed widely to accelerate neuroscience and reduce the number of people suffering from brain disorders. To do so, we combine rigorous bioengineering with an expertise in biotechnology entrepreneurship that is unique among academic labs, allowing us to identify more important unmet needs and deploy solutions more rapidly. Examples of previous projects include the development of Implosion Fabrication [1], a bio-inspired 3D nanofabrication method; Slide-seq [2], a spatial transcriptomic technique that enables researchers to visualize the expression of every gene in the genome simultaneously; and Timestamps [3], a method that allows the age of RNAs to be deduced from RNA sequencing.

The ABL is seeking a clinical fellow to work on building new viral vectors for gene therapy in the brain. Many brain disorders, such as Fragile-X and spinal muscular atrophy (SMA), are monogenic, stemming from a single well-defined mutation in the genome. For these disorders, there is hope that they could be cured using gene therapies, but delivering the replacement gene to the targeted cells is a major challenge. Luxturna and Zolgensma, the first two approved in vivo gene therapies, both rely on adeno-associated viruses (AAVs) as vectors for gene delivery, but AAVs only carry relatively small gene payloads, whereas many prospective gene therapies (such as many of those that use CRISPR) would require delivery of much larger genes. Moreover, most AAVs do not cross the blood-brain barrier, and must be surgically injected into the target tissue. Luxturna, for example, is delivered by direct injection into the eye, whereas the AAV gene therapies for Parkinson's disease by Voyager Therapeutics are injected through a hole drilled in the skull while the patient lies inside an MRI.

We are seeking to develop a new generation of AAV viral vectors with improved tropism, better immune evasion, a larger packing size, and a greater ability to cross the blood-brain-barrier. The exact project will be determined upon joining, and lab members are encouraged to come up with their own ideas. In general, we expect to leverage our deep expertise in high-throughput screening to identify AAV variants with more desirable properties, and will then take advantage of a broad network of collaborators to test our new vectors in a variety of in vitro and animal models. Ultimately, the clinical fellow will also prototype the new vectors in human brain tissue, through collaboration with clinical co-advisors at UCL. If the project is successful, the fellow will also have the opportunity to participate in licensing or co-found a company developing the technology for clinical applications.

The partner institution for this project is UCL.

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Identifying and functionally characterising non-coding drivers of renal cancer evolution

A PhD project for the 2021 doctoral clinical fellows programme with Samra Turajlic (primary supervisor, Crick) and Richard Houlston (The Institute of Cancer Research)

Clear Cell Renal Cell Cancer (ccRCC, accounting for 75% of all RCCs) is characterised by frequent mutation of a small set of driver genes (e.g. VHL, PBRM1, SETD2, BAP1), with a number of rarer mutational drivers (e.g. KDM5C, MTOR, PTEN). Patient outcome from ccRCC is variable, and while the timing and combination of driver mutations accounts for some of the diversity, the full complement of molecular lesions underpinning this disease is unknown.

The importance of non-coding and copy number driver mutations in cancer is increasingly being recognised. Different classes of non-coding driver mutation have been described including rearrangements juxtaposing highly-expressed promoters and oncogenes, promoter/enhancer mutations, and changes in chromatin structure creating aberrant topologically associating domains. Efforts are being directed to systematically analyse the non-coding genome for cancer-driving mutations for a number of cancers. Cis-regulatory regulatory elements (CREs) influencing gene expression represent a highly-enriched subset of the non-coding genome in which to identify non-coding drivers. The aim of the project is to define the regulatory landscape of RCC by using high-throughput chromosome conformation capture (Hi-C) and to map CREs. Integrating this information with whole genome sequence data from the 100,000 (100K) Genomes Project (https://www.genomicsengland.co.uk/the-100000-genomes-project/) and TRACERx Renal study (http://tracerx.co.uk/studies/renal/) to identify and characterise non-coding drivers for RCC. Recent evolutionary classification of clear cell RCC which reconciles some of its clinical diversity (Turajlic et al. 2018a, 2018b) will be refined using the non-coding genome alterations to understand how they constrain cancer evolution. Finally, germline variation (defined from the 100K and TRACERx Renal cohorts) will be also be evaluated as a potential source of evolutionary constraint.

The partner institution for this project is The Institute of Cancer Research.

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Evaluating amino acid-based PET tracers to predict metabolic sensitivities in breast cancers

A PhD project for the 2021 doctoral clinical fellows programme with Mariia Yuneva (primary supervisor, Crick), Ashley Groves (UCL), Stefan Voo (UCL) and Neill Patani (UCL)

The role of metabolic reprogramming in different aspects of tumorigenesis is now well established. The requirement of various metabolic pathways has been demonstrated for proliferation and survival of tumour cells as well as for the maintenance of their microenvironment (1). Although multiple studies proposed inhibiting various metabolic pathways as potential anti-cancer strategies, targeting metabolism still proves to be challenging due to genetic and metabolic heterogeneity of tumours and flexibility of metabolism (1,2). The overarching goal of our programme is to uncover how specific genetic lesions determine metabolic profiles of tumours and their metabolic requirements, and how these relationships are shaped by tissue context and tumour microenvironment. We are identifying the mechanisms of metabolic plasticity of tumours including an interplay between different metabolic pathways and metabolic interactions between a tumour and a host. Ultimately, we are seeking to identify more efficient therapeutic strategies aimed at targeting metabolic pathways at different stages of tumorigenesis bound to specific oncogenic lesions.

One of the most dysregulated lesions in various types of tumours is a transcriptional factor proto-oncogene MYC. We and others have demonstrated that MYC regulates multiple metabolic activities and makes tumour cells sensitive to specific metabolic perturbations (3). Using mouse models in combination with metabolomics approaches we have identified metabolic pathways of central carbon metabolism supporting formation and growth of tumours driven by MYC. We have also demonstrated how pathways defining metabolic plasticity of tumours can be identified and targeted to improve a therapeutic outcome of metabolism-based therapies (3). One of the main challenges, however, of translating the results we and others have obtained in preclinical models of tumourigenesis to the clinic is to identify patients who may benefit from specific metabolism-targeting therapies.

Positron emission tomography (PET) tracers based on aminoacids, including *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine (18F-FET), 6-[^{18}F]Fluoro-L-DOPA ([^{18}F]F-DOPA) and anti-1-amino-3-^{18}F-fluorocyclobutane-1-carboxylic acid (^{18}F-FACBC) have been used in preclinical models and clinic to image different types of tumours (4). These tracers are mostly transported by amino acid transporters ASCT2 (Slc1a5) and LAT1 (Slc7a5). Both of the transporters are upregulated in multiple types of cancers, are known to be transcriptionally regulated by MYC (5) and demonstrate increased expression in MYC-induced tumours in mouse models. Both of the transporters are linked to metabolic pathways of glutamine and other amino acids as well to signalling pathways including mTOR pathway (5). The work by our laboratory and others demonstrated that these pathways can be targeted for the therapy of tumours with dysregulated MYC activity (3). The current project proposes to evaluate the use of amino acid-based PET tracers in preclinical models of MYC-induced tumourigenesis as markers of increased transporter activity and sensitivity to metabolism-based inhibitors associated with high MYC activity. We will also combine PET imaging studies with mass spectrometry imaging (MSI) to evaluate the heterogeneity of metabolic activities and therapeutic responses within a tumour. We will next evaluate how PET signals correlate with MYC activity and metabolic requirements in patient derived models of breast cancer. Finally, using genetic and metabolic analysis of PET-guided tumour tissue biopsies we will evaluate these relationships in cancer patients.

The partner institution for this project is UCL.

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