# Theme 1

# Molecules, Cells and the Basis for Disease

# 2021/2022

## Molecules, Cells and the Basis for Disease

This theme brings together stem cells and regenerative medicine (inc. cellular therapies), immunology, genetics, cellular biology (particularly relating to cancer), and biophysics. These areas – and particularly the interfaces between them – are current strengths and priorities for King's.

Lead: Professor Rebecca Oakey & Dr Cynthia Andoniadou

When choosing a project from this catalogue in the funding section & research proposal section of the online application form please enter MRCDTP2021_Theme1

Deadline for application: Sunday 29th November 2020

Shortlisted candidates will be contacted in early January.

Interviews: Wednesday 27th and Thursday 28th January 2021

The 2021/22 studentships will commence in September 2021.

For further Information or queries relating to the application process please contact mrc-dtp@kcl.ac.uk

Projects listed in this catalogue are subject to change, candidates invited to interview will have the opportunity to discuss projects in further detail.

## Contents

## 1.1 Defining how to prevent immune activation due to transient nuclear envelope ruptures.

Co Supervisor 1A: Monica Agromayor
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**Project Description:**

The innate immune system is activated by specialized pattern recognition receptors (PRR), which. recognise specific structures that are unique or otherwise absent in the subcellular location where they are detected. Compartmentalization of genomic DNA in the nucleus is maintained by the nuclear envelope (NE) and is considered essential to avoid aberrant innate immune activation by cytoplasmic DNA sensors like cyclic GMP-AMP synthase (cGAS) and its downstream signalling effector stimulator

of interferon genes (STING). Importantly, recent observations that cGAS is also present in the nucleus and the discovery of hnRNPA2B1, a nuclear viral DNA sensor, suggest that a safeguard mechanism, in addition to physical separation, may be necessary to help differentiate pathogenic from self-DNA.

Cancer cells and immune cells can migrate through dense tissues and constricted spaces and experience *n*uclear *e*nvelope *r*upture *d*uring *i*nterphase (NERDI). These rupture events cause mislocalisation of the nuclear content, inducing expression of interferon-stimulated genes (ISGs) via activation of the cGAS–STING pathway. Thus, NERDI events need an "immunologically silent" resolution and repair process. Recent studies have identified several cellular components required for NE re-sealing, including the endosomal sorting complex required for transport (ESCRT) machinery, integral membrane proteins of the Lap2-emerin-MAN1 (LEM) family and the protein barrier-to-autointegration factor (BAF).

This project will explore how ESCRT factors may regulate signalling by cytoplasmic DNA sensors in the context of NERDIs. Specifically, the protection provided by these mechanisms to regulate sensing of self-DNA after rupture of the nuclear envelope will be determined. The student will address these questions using biophysics, cutting edge microscopy and molecular biology techniques.

**One representative publication from each co-supervisor:**

Agromayor & Martin-Serrano. *Trends Cell Biol*. (2013) DOI: 10.1016/j.tcb.2013.04.006

Y. Zhao *et al*. *Nature Communications* (2018) DOI:10.1038/s41467-018-06089-1

## 2.1 Non-viral Delivery of Nucleic Acids using Nanomedicines for Cardiac Regeneration.

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Co Supervisor 1B: Mauro Giacca
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**Project Description:**

A growing number of patients surviving myocardial infarction suffer from permanent damage of the heart, which frequently leads to heart failure. This condition is common, lethal and expensive to treat long-term. Available small molecule drugs prescribed for heart failure aim at improving residual cardiac performance, while non counteracts the loss of cardiomyocytes. This studentship aims at developing biological treatment option which will stimulate endogenous cardiomyocytes proliferation after myocardial infarction (MI). Prof Giacca's team (co-supervisor) has identified a human microRNA that is effective in stimulating cardiomyocytes proliferation. They have shown that viral expression of

this specific microRNA using viral vectors stimulated cardiomyocytes proliferation in mouse and pig models. Viral delivery of nucleic acids although effective suffers from severe side effects so the search for safer yet effective delivery options constitutes an unmet need. The team of Prof Al-Jamal (cosupervisor) has developed a range of non-viral vectors e.g. polymer-, carbon- and lipid-based nanoparticles, particularly suited for the delivery of nucleic acids (siRNA, mRNA and pDNA) to treat a range of diseases including cancer and neurodegenerative diseases. This project aims to develop a lipid-based nanocarrier system suitable for the delivery of therapeutic nucleic acids following systemic administration. The project will be divided into four main stages: (i) the formulation and characterisation of actively targeted nanomedicines containing nucleic acids; (ii) *in vitro* transfection and safety assessment of the developed carriers; (iii) *in vivo* optical imaging to confirm targeting the infarcted area in MI mouse model; (iv) therapeutic efficacy studies in MI mouse model.

**One representative publication from each co-supervisor:**

Gabisonia K, Prosdocimo G, Aquaro GD, Carlucci L, Zentilin L, Secco I, Ali H, Braga L, Gorgodze N, Bernini F, Burchielli S, Collesi C, Zandonà L, Sinagra G, Piacenti M, Zacchigna S, Bussani R, Recchia FA, Giacca M. *Nature*. 2019 May; 569(7756):418-422. doi: 10.1038/s41586-019-1191-6.

Xu L, Faruqu FN, Lim YM, Lim KY, Liam-Or R, Walters AA, Lavender P, Fear D, Wells CM, Wang J T-W, Al-Jamal KT. *Biomaterials*. 2020 Sep. https://doi.org/10.1016/j.biomaterials.2020.120369

### 3.1 Metabolic control of cell fate commitment in the pituitary.

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Co Supervisor 1B: Zoe Mann
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**Project Description:**

The pituitary is a primary endocrine gland controlling fundamental physiological functions. It contains hormone-secreting cells, which are derived from pituitary stem cells (PSCs) throughout life. Diseases of the gland, such as hypopituitarism, can affect one or multiple of these cells and could benefit from regenerative medicine approaches. A barrier to translation, is our inability to direct stem cells down appropriate commitment steps.

Using single cell RNA-seq of purified PSCs, we have identified 5 unique clusters, representing varying commitment stages. During commitment, stem cells have different energy requirements, leading to metabolic changes. These can be reflected through differences in mitochondrial morphology, mitochondrial membrane potential, and whether a cell utilises glycolytic or oxidative pathways. This metabolic re-programming regulates transcription programs specifying cell fates. Therefore, driving distinct metabolic pathways at key lineage branch points may facilitate directing progenitor fate.

Overall objectives:

1) *Establish the metabolic phenotype of pituitary stem cell states at the transcriptomic level.* Analyse existing scRNA-seq datasets to identify key metabolic genes and transcription factors associated with pathway utilisation at different stages of PSC commitment.

2) *Generate a map of changes in the metabolomic landscape during pituitary stem cell commitment.* Utilising a number of established live imaging assays to assess metabolism in vitro and ex vivo to compare metabolic profiles between different cell states.

3) *Manipulate pituitary stem cell fate through metabolic regulation in vitro and in vivo.* Determine the effects of perturbed metabolism on cell state and lineage selection in mouse and human PSCs using metabolic inhibitors to perturb either glycolysis or oxidative phosphorylation.

Key techniques: bioinformatics, microscopy, metabolic assays, primary cell culture, organ/tissue slice culture, mouse work.

**One representative publication from each co-supervisor:**

Homeostatic and tumourigenic activity of SOX2+ pituitary stem cells is controlled by the LATS/YAP/TAZ cascade. Lodge EJ, Santambrogio A, Russell JP, Xekouki P, Jacques TS, Johnson RL, Thavaraj S, Bornstein SR, Andoniadou CL. *Elife*. 2019 Mar 26;8:e43996. doi: 10.7554/eLife.43996

Characterization of the development of the mouse cochlear epithelium at the single cell level. Kolla L, Kelly MC, Mann ZF, Anaya-Rocha A, Ellis K, Lemons A, Palermo AT, So KS, Mays JC, Orvis J, Burns JC, Hertzano R, Driver EC, Kelley MW. *Nat Commun.* 2020 May 13;11(1):2389. doi: 10.1038/s41467-020-16113-y

## 4.1 Investigation into novel immunotherapy candidates for modulating the anti-tumour immune response.

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**Project Description:**

This interdisciplinary project combines the preclinical *in vivo* expertise studying the tumour microenvironment, macrophages and the anti-tumour immune response of the Arnold lab and the medicinal chemistry expertise of the Rahman lab. This project will explore and develop novel immunotherapy candidates which enhance the anti-tumour immune response through modulating the immune suppressive capabilities of tumour associated macrophages. One target of interest is the enzyme heme oxygenase-1 which plays a pivotal role in immune suppression in the tumour. The project will both develop and optimise drug candidates using synthetic chemistry approaches and *in vitro* assays that will be designed to test activity and selectivity. These compounds will then be explored using preclinical models of cancer for anti-tumour efficacy. *Ex vivo*, tumour tissue will be investigated to explore the mechanistic basis for any anti-tumour control observed. There will be a particular focus on monitoring for immune-modulatory effects of the drug candidates which will be explored using rigorous immune phenotyping of the microenvironment. It is anticipated that the project could generate novel drug candidates with potential for eventual translation to the clinic. The project is anticipated to involve; *in vivo* models of cancer, flow cytometry, confocal microscopy, transcriptomics, cell culture, *in vitro* drug testing, chemical engineering and *in silico* screening/modelling and mass spectroscopy.

Overarching objectives;

1) Identify/optimise novel candidate immunotherapy drugs using molecular modelling.

2) Investigate the anti-tumour efficacy of the candidate drug(s) using *in vivo/vitro* models of cancer.

3) Characterise the immunomodulatory capabilities of the candidate drug(s) and mechanism of tumour control.

**<u>One</u> representative publication from <u>each co-supervisor</u>:**

Muliaditan, T., Caron, J., Okesola, M., Opzoomer, J.W., Kosti, P., Georgouli, M., Gordon, P., Lall, S., Kuzeva, D.M., Pedro, L., Shields, J.D., Gillett, C.E., Diebold, S.S., Sanz-Moreno, V., Ng, T., Hoste, E. and

Arnold, J.N. (2018) Macrophages are exploited from an innate wound healing response to facilitate metastases in cancer. *Nature Communications*, 9:2951.

Corcoran, D. B., Lewis, T., Nahar, K. S., Jamshidi, S., Fegan, C., Pepper, C., Thurston, D. E., and Rahman, K. M. (2019) Effects of Systematic Shortening of Noncovalent C8 Side Chain on the Cytotoxicity and NF-kappa B Inhibitory Capacity of Pyrrolobenzodiazepines (PBDs), *Journal of Medicinal Chemistry* 62, 2127-2139.

## 5.1 Investigating the unique properties of the oligodendrocyte microtubule cytoskeleton in myelin growth, degeneration and repair.

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**Project Description:**

Neuronal axons in the central nervous system are insulated in multiple layers of fatty material know as myelin, produced by glial cells called oligodendrocytes (OLs), ensuring fast and efficient signal transmission. Insufficient myelination is associated with 'myelinopathies' including multiple sclerosis and leukodystrophies. Augmented remyelination is a promising therapeutic strategy in myelinopathies.

Myelination is driven by OL cytoskeleton remodelling, including the reorganisation of dynamic microtubules under the control of regulatory microtubule-associated proteins (MAPs). To this end, OLs express specific microtubule tubulin isoforms and MAPs with specialised functions, yet their structure, function and dynamics are poorly understood. Mutations in the OLspecific βIV tubulin isoform lead to a rare leukodystrophy with myelin deficiency. Characterisation of OL-specific microtubules and MAPs will help us understand their roles in myelination, demyelination and remyelination.

The student will use a combination of the exciting structural techniques cryo-electron microscopy (cryo-EM) and nuclear magnetic resonance (NMR) and biochemical and biophysical methods to investigate the structure, dynamics and function of the βIV tubulin isoform and disease-causing mutations as well as various MAPs and their interactions with microtubules. Expert training in these methods will be provided by the supervisors and their respective groups. In year 1, the student will focus on training, begin purifying MAPs for investigation and characterise wild-type and mutant βIV tubulin. In year 2 the student will characterise MAP interactions with and effects on microtubules. In year 3, the student will continue year 2 objectives, with more focus on data analysis and thesis write up.

**<u>One</u> representative publication from <u>each co-supervisor</u>:**

Atherton, J. et al. A structural model for microtubule minus-end recognition and protection by CAMSAP proteins. *Nat. Struct. Mol. Biol*. 24, 931–943 (2017).

Cruz-Gallardo, I., Martino, L., Kelly, G., Atkinson, A., Trotta, R., De Tito, S., Coleman, P., Ahdash, Z., Gu, Y., Bui, T.TT Conte, M.R. (2019) LARP4A recognises polyA RNA via a novel binding mechanism mediated by disordered regions and involving the PAM2w motif, revealing interplay between PABP, LARP4A and mRNA. *Nucleic Acids Res., 47:4272-4291*

## 6.1 Metabolic regulation of immune responses during infection.

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**Project Description:**

Toll-like receptors (TLRs) are pattern recognition receptors present on many immune cells that sense microbial invasion and ultimately control immune responses during viral and bacterial infection. In macrophages, TLR ligation leads to the production of cytokines that are crucial to induce productive immunity, leading to pathogen clearance. Conversely, cytokine over-production, resulting in a "cytokine storm", can be harmful in many patho-physiological conditions such as acute viral infections, sepsis and autoimmune diseases. Thus, the strict control of the pathways leading to TLR activation is critical for the regulation of immune responses. A key mechanism that is instrumental in the regulation of TLR signalling is cellular metabolism. Accordingly, alterations in lipid metabolism induce the secretion of cytokines by macrophages and aid in anti-viral immunity. On the other hand, viral and bacterial infections lead to alterations in the host's lipid homeostasis. Thus, targeting lipid metabolism in immune cells can help to eliminate infection, yet our knowledge of this immunometabolic circuit is still very limited.

Project Aim: This project intends to uncover the mechanisms by which cellular metabolism controls immune responses during infection.

Objectives:

Year 1. We will use a combination of next-generation sequencing and lipidomics to identify the molecular links between TLR signalling and lipid metabolism pathways in immune cells.

Year 2. We will take advantage of *in vitro* cultures and cellular and molecular biology techniques to manipulate lipid metabolism pathways and determine their effect on immune responses.

Year 3. We will use transgenic mice and *in vivo* models of infection to uncover the link between lipid metabolism and pathogen clearance. Skills training include cell culture, flow-cytometry, standard molecular biology and cellular immunology techniques, RNA-seq, animal work, CRISPR/Cas9 technology.

**One representative publication from each co-supervisor:**
*Initiation of Antiviral B Cell Immunity Relies on Innate Signals from Spatially Positioned NKT Cells*
Mauro Gaya, Patricia Barral, Marianne Burbage, Shweta Aggarwal, Beatriz Montaner, Andrew Warren Navia, Malika Aid, Carlson Tsui, Paula Maldonado, Usha Nair, Khader Ghneim, Padraic G Fallon, Rafick-Pierre Sekaly, Dan H Barouch, Alex K Shalek, Andreas Bruckbauer, Jessica Strid, Facundo D Batista. Cell (2018) DOI: 10.1016/j.cell.2017.11.036

*RNF144A shapes the hierarchy of cytokine signaling to provide protective immunity against influenza*.

Afzali, S. Kim, E. West, E. Nova-Lamperti, N. Cheru, H. Nagashima, B. Yan, T Freiwald, N. Merle, D. Chauss, M. Bijlmakers, G. Weitsman, Z. Yu, D. Jankovic, S. Mitra, A. Villarino, C. Kemper, A. Laurence, M. Kazemian, J.J. O'Shea, S. John. BioRXiv.(2019) https://doi.org/10.1101/782680

## 7.1 Structural characterization of the anti-HIV protein MX2 and its interactions with viral and cellular factors.

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**Project Description:**

MX2 is a potent cellular antiviral protein, and a component of innate immunity. It is particularly active in preventing HIV replication.

The antiviral activity of MX2 has been associated with two distinct mechanism: (1) MX2 binds to the HIV capsid (CA), and prevents its ordered disassembly, required for viral replication; and (2) it interacts with the nucleopore, and prevents the entry of the viral genome (as well as other cargo) into the cell nucleus. Recent unpublished data have also revealed that in the absence of viral infection MX2 is phosphorylated, which prevents its association with the nucleopore and alleviates its cytotoxic effect.

Critically, the molecular basis for these mechanisms is not currently understood.
The structure of MX2 has been reported previously. However, there is little understanding of the structural basis for its interaction with CA, the nucleopore, kinases, or phosphatases. In particular, the N-terminal domain, which is essential for antiviral function, is not resolved in any of the published structures to date.

For this project, we propose a structure-function analysis of MX2's interaction with its binding partners. A range of biophysical and biochemical techniques (SEC-MALS, ITC, NMR) will be employed to characterize these interactions, and the structures of the complexes will be determined by cryoEM or X-ray crystallography. Finally, cell-based assays will be employed to assess how these interactions regulate MX2' antiviral activity and cytotoxicity. Collectively, this work will provide fundamental knowledge on this recently discovered host-pathogen interaction and form a foundation for future exploitation in the development of new therapeutics.

**One representative publication from each co-supervisor:**

*The GTPase Domain of MX2 Interacts with the HIV-1 Capsid, Enabling Its Short Isoform to Moderate Antiviral Restriction*. Betancor G, Dicks MDJ, Jimenez-Guardeño JM, Ali NH, Apolonia L, Malim MH. Cell Rep. 2019 Nov 12;29(7):1923-1933.e3. doi: 10.1016/j.celrep.2019.10.009.

*The cryo-EM structure of the bacterial flagellum cap complex suggests a molecular mechanism for filament elongation*. Al-Otaibi NS, Taylor AJ, Farrell DP, Tzokov SB, DiMaio F, Kelly DJ, Bergeron JRC. Nat Commun. 2020 Jun 25;11(1):3210. doi: 10.1038/s41467-020-16981-4.

## 8.1 How does HLA-B27 cause ankylosing spondylitis?

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**Project Description:**

Ankylosing spondylitis (AS) is a common chronic immune-mediated arthritis affecting primarily the spine and pelvis. The disease is strongly associated with the gene *HLA-B27* and is polygenic. Amongst the other known associated genes are two aminopeptidases, *ERAP1* and *ERAP2*, whose canonical function is to trim antigenic peptides prior to presentation by HLA Class I antigens such as HLA-B27 to T-cells. The *ERAP1* association with AS is restricted to HLA-B27+, or HLA-B27/HLA-B40+ cases. The same variants are also associated with psoriasis and Behcet's disease; *ERAP2* is associated with inflammatory bowel disease.

The mechanisms by which HLA-B27 induces AS, and how ERAP1/2 influence this, are unclear. We have generated transgenic mice carrying human *ERAP1* or *ERAP1/2*, and mice lacking these genes, which have been cross-bred onto a HLA-B27/beta-2-microglobulin line.

This project aims to investigate how HLA-B27 causes AS, testing key hypotheses by comparing findings in different mouse strains, and using pharmacological approaches using ERAP inhibitors.

In years 1-2 peripheral blood and stool samples will be collected from different lines. Flow cytometry will be used to measure HLA-B27 isoform expression and single-cell sequencing employed to quantify cell populations and gene-expression differences between strains. In years 2-3, T-cell repertoire profiling of CD4 and CD8 lymphocytes will be performed, using next-generation sequencing. In year 3, 16S rRNA profiling will be used to investigate host genotype effects on stool microbiome. To establish the relevance of findings obtained in mice to human disease, murine data will be compared with data generated in AS patients in years 3-4.

**<u>One</u> representative publication from <u>each co-supervisor</u>:**

*Genetic variants in ERAP1 and ERAP2 associated with immune-mediated diseases influence protein expression and isoform profile*. Hanson A, Cuddihy T, Haynes K, Loo D, Morton CJ, Opperman U, Leo P, Thomas GP, Le Cao KA, Kenna TJ, Brown MA. *Arthritis Rheum,* 70(2):255-265, 2018.

*T-cell phenotyping uncovers systemic features of atopic dermatitis and psoriasis*. Farrera C, Melchiotti R, Petrov N, […] Di Meglio P. *J Allergy Clin Immunol*. 145(3):1021-1025.e15. 2020

## 9.1 Studying the regulation of cardiovascular metabolism by novel redox-dependent processes.

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**Project Description:**

As cardiovascular disease is a leading cause of death, it is imperative we improve our understanding of the underlying biological processes that contribute to its pathogenesis. Both increased oxidant formation and changes in cellular metabolism are widely associated with the development of cardiovascular disease. Therefore, we hypothesise that a novel molecular process may link thiol oxidation to pathological changes in cellular metabolism. This is based on the oxidation of susceptible cysteine thiols within select metabolic enzymes, which will alter their function through reversible disulphide formation. In this study candidate metabolic proteins identified from a novel structural bioinformatic screen, will be assessed for redox-dependent reversible disulphide formation, and the functional impact of this process characterised using a range of cell biology, molecular, physiological and biophysical techniques. Here expert training in studying and characterising protein oxidation will be provided by Dr Joseph Burgoyne, while expertise in cardiac metabolism and physiology will be provided by Prof Michael Shattock. It is anticipated that findings from this study will lead to the identification of central metabolic enzymes that can undergo redox-dependent disulphide formation, modifications that will have important implications in regulating cardiovascular health. Also, importantly findings are likely to identify new sites that can be directly targeted for therapy. This is supported by our previous work, where we identified thiol reactive compounds that can modify a susceptible cysteine in the cGMP-dependent protein kinase 1a, which leads to lowering of blood pressure, and with further development could be used as a therapy.

**One representative publication from each co-supervisor:**

Prysyazhna O, Wolhuter K, Switzer C, et al. Blood Pressure-Lowering by the Antioxidant Resveratrol Is Counterintuitively Mediated by Oxidation of cGMP-Dependent Protein Kinase. *Circulation*. 2019;140(2):126-137

Aksentijević D, Karlstaedt A, Basalay MW, et al. Intracellular sodium elevation reprograms cardiac metabolism. *Nature Communications*. 2020; 11: 4337

## 10.1 Exploring the mechanisms of beta cell expansion during pregnancy using single cell transcriptomics.

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**Project Description:**

In late pregnancy the foetus is growing rapidly, and the consequent demand for nutrients must be met by maternal physiological adaptations. These adaptations are brought about by hormonal signals from the conceptus. In the second half of pregnancy maternal insulin resistance increases due to placental hormone production, and this prioritises the transfer of glucose across the placenta, diverting it towards foetal growth. In response to this, the mother increases her pancreatic beta cell mass so she can produce more insulin. Whilst this physiological process is well described in humans and rodents, the molecular mechanisms by which maternal beta cell expansion occurs are not fully understood. This is important for several reasons; i) failure of maternal beta cell expansion causes gestational diabetes mellitus (GDM), a hyperglycaemic crisis that threatens the life of both mother and baby and ii) new mechanisms that can promote beta cell expansion have considerable clinical potential to combat increasing rates of Type II Diabetes.

The student will learn to combine manipulation of murine models of pregnancy with cutting edge techniques in single cell transcriptomics to answer the following questions:

1. How does the beta cell compartment expand during pregnancy?
2. Is there evidence of neogenesis of beta cells from a yet undiscovered stem cell population?
3. Is this expansion compromised in obese mothers?

Years 1-2 will develop the pregnancy model, perform single cell transcriptomics on beta cells, analyse the data and validate targets. Year 3 will extend these findings into a model of maternal obesity.

**One representative publication from each co-supervisor:**

Cleaton MAM, Corish JA, Howard M, Gutteridge I, Takahashi N, Bauer SR, Powell TL, Ferguson-Smith AC, Charalambous M. (2016). Conceptus-derived Delta-like homologue-1 (DLK1) is required for maternal metabolic adaptations to pregnancy and predicts birthweight. *Nat Genet* 48(12):1473-1480.

Sancho R, Gruber R, Gu G, Behrens A. Loss of Fbw7 reprograms adult pancreatic ductal cells into α, δ, and β cells. *Cell Stem Cell*. 2014 Aug 7;15(2):139-53.

## 11.1 Emergent matrix properties from the nano to macro scale.

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**Project Description:**

Tissue fibrosis is associated with a variety of diseases, from cancer to pathological scarring, and involves a dramatic change in extracellular matrix (ECM) mechanics (e.g. stiffness and viscosity). The ECM is a biopolymer network produced by fibroblasts, which both create andremodel the network. In this project we will investigate the molecular and mechanical changes associated with a fibrotic ECM in an in vitro model of fibroblast scar formation. It is currently unknown whether the increase in ECM stiffness arises from stiffening of individual ECM fibres, a change in the degree of crosslinking, or another organisational effect. We will image fibroblast-generated ECM at superresolution and test its properties using atomic force microscopy (AFM). To complement the experimental data we will create a detailed model of the ECM network, including fibre stiffness and cross-linking. Modelling will give us insight into how the macro-scale stiffness is influenced by changes in the nanoscale properties.

The student involved with this project will get exposure to a variety of imaging techniques, from superresolution microscopy to AFM, along with cell culture and molecular biology. Depending on the background of the candidate, they may also become familiar with deep learning approaches and finite element modelling (note that programming is not a prerequisite for this project).

Objectives:
Year 1 - develop imaging approaches to measure mechanical properties of a fibroblast-derived ECM.
Year 2 - compare and contrast normal and fibrotic ECM networks.
Year 3 - develop modelling approaches to understand how the mechanics arise in the system.

**One representative publication from each co-supervisor:**

Marsh et al., Artifact free high density localisation analysis, 2018 Nature Methods

Matsubayashi et al., Rapid Homeostatic Turnover of Embryonic ECM During Tissue Morphogenesis. 2020 Dev Cell

### 12.1 Understanding and harnessing the regenerative properties of apoptotic cells.

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**Project Description:**

Mesenchymal stromal cells (MSCs) exert potent anti-inflammatory and tissue repair activities successfully tested in different clinical conditions. We have recently shown that these properties require MSC to undergo *in vivo* apoptosis. Apoptotic MSCs (apoMSCs) are ultimately responsible for the immunosuppressive activity by reprogramming tissue macrophages. Furthermore, caspase activation upregulates several other molecules that control inflammation.

Hypothesis: apoMSCs control injury-induced acute inflammation and promote wound healing. The student will characterise the cellular and molecular machinery involved in delivering the MSC apoptotic signal that conveys the anti-inflammatory and regenerative activities.

Year 1-2: Identify the molecular profile of apoMSCs by RNAseq and validate the critical molecules using in vitro systems. The molecular profile will be tested for the ability to affect epithelial cell and dermal fibroblast proliferation/migration/survival and activation/scarring readouts.

Year 3-4: Test the ability of apoMSCs and their supernatant to promote wound healing in an animal model. In addition to assessment of wound outcomes (e.g. speed of closure, scarring), the evaluation will include extensive tissue analysis of the skin and draining lymph nodes in order to enumerate and functionally characterise the macrophage populations recruited at the site of injury. The system will be eventually used to test the relevance of the apoMSC molecular repertoire to replace the use of cells. This will be done by gain of function approach with available molecules/drugs and loss of function approach using CRISPR-Cas9 editing.
Both supervisors have extensive expertise in the immunobiology of tissue repair, technology and animal models are in the portfolio of both laboratories.

**One representative publication from each co-supervisor:**

Galleu A, Riffo-Vasquez Y, Trento C, …and Dazzi F. Perforin-dependent apoptosis in mesenchymal stromal cells is required to initiate host-mediated *in vivo* immunomodulation. Science Transl Med 2017 *vol 9, Issue 416, eaam7828*

Barallobre-Barreiro J, Woods E, Bell RE, Easton JA, Hobbs C, Eager M, Baig F, Mackenzie Ross A, Mallipeddi R, Powell B, Soldin M, Mayr M, Shaw TJ. *Cartilage-like composition of keloid scar extracellular matrix suggests fibroblast mis-differentiation in disease.* Matrix Biol Plus, 2019. 4: 100016.

## 13.1 Identification of genomic mechanisms of rare disease using multidimensional data integration.

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**Project Description:**

The “Genomics revolution” has transformed the way we practice and envision clinical medicine, particularly within the field of rare diseases. The identification of disease genes associated with rare phenotypes has yielded important biological information relevant to disease and development, but also with regards to the mechanisms underlying more common human disorders.
However, these developments are just beginning, and their full potential remains untapped. Genomics England has pioneered the implementation of genomic technologies in the healthcare setting, yet over half of patients with rare diseases remain undiagnosed. Multidisciplinary clinical academic centres present an ideal collaborative environment where researchers and clinicians can approach this pressing problem collaboratively and effectively by harnessing existing genomic and phenotypic data, and applying innovative research methods. The project aims to identify novel genomic mechanisms of disease by taking a personalised medicine approach to a cohort of individuals with rare disease, harnessing genomic data and collaborations through Genomics England Clinical Interpretation Partnerships and other large scale omics dataset.

The student will undergo training in computational biology and genomic analysis and scientific writing.

Objectives:
Year 1 - Using 100,000 and National Genomic Research Library genomes, optimise analytical tools for the identification of non-coding genomic variation in rare disease individuals.
Year 2 - Apply optimized tools for reanalysis of research and diagnostic genomic data of a cohort of deep phenotyped individuals with rare disease. Analyse transcriptomic data from the same cohort.
Years 3-3.5 - Develop analytical methods for multidimension integration of genomic, transcriptomic and clinical data for identification of novel disease mechanisms.

**<u>One</u> representative publication from <u>each co-supervisor</u>:**

Petridis C, Navarini AA, Dand N, Saklatvala J, Baudry D, Duckworth M, Allen MH, Curtis CJ, Lee SH, Burden AD, Layton A, Bataille V, Pink AE; Acne Genetic Study Group, Carlavan I, Voegel JJ, Spector TD, Trembath RC, McGrath JA, Smith CH, Barker JN, Simpson MA. Genome-wide meta-analysis implicates mediators of hair follicle development and morphogenesis in risk for severe acne. *Nat Commun*. 2018.

Dias C, Busquetes Estruch S, Graham SA, McRae J, Sawiak SJ, Hurst JA, Joss S, Holder SE, Morton JEV, Turner LSC, Thevenon J, Mellul K, Sanchez-Andrade G, Deriziotis P, Santos RF, Ibarra-Soria X, Lee SC, Faivre L, Kleefstra T, Liu P, Hurles ME, the DDD Study, Fisher SE & Logan DW. BCL11A haploinsufficiency causes an intellectual disability syndrome and dysregulates transcription. *Am J Hum Genet.* 2016, Aug 4;99(2):253-274.

## 14.1 Identification of molecular mechanisms underlying response and resistance to BCL-2 inhibition in NPM1 mutated Acute Myeloid Leukaemia by multi-omic characterisation of patients in the VICTOR trial.

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**Project Description:**

Acute myeloid leukaemia (AML) is a common, aggressive and frequently fatal blood cancer affecting both children and adults. Current treatments involve high-dose chemotherapy which causes significant morbidity and mortality and only cures a minority of patients. A promising alternative treatment is activation of apoptosis by small-molecule inhibition of BCL-2. Early clinical studies suggest durable remission is restricted to specific genotypes, notably *NPM1*$^{mutant}$/*FLT3*ITD$^{negative}$ (accounting for ~25% of patients). Our group is leading a clinical trial comparing BCL-2 inhibition to chemotherapy in this group; we observe a spectrum of responses from complete resistance to deep molecular remission indicating that genotype alone fails to fully predict susceptibility.

This project will exploit a multi-omics approach to deeply characterise patients with either good or poor response to BCL-2 inhibition allowing identification of novel biomarkers predictive of outcome, and providing insights into mechanisms of drug resistance, which may themselves be targetable.

- The student will perform whole-transcriptome and ATAC sequencing using primary patient samples and will integrate these results with whole genome sequence data and with clinical outcomes (year 1-2).
- For patients with residual or recurrent disease, single-cell methodologies (10x and TARGETseq) will be applied to paired diagnosis and post-treatment samples to define gene expression profiles which characterise drug resistant populations (year 2-3).
- Candidate markers of resistance will be validated and further investigated using appropriate techniques which are likely to include cut&tag, CRISPR, western blot and drug sensitivity assays (year 3-4).

Full training for all laboratory and computational aspects of the work will be provided as needed.

**<u>One</u> representative publication from <u>each co-supervisor</u>:**

Dillon R, Hills RK, Freeman S, et al (2020) Molecular MRD status and outcome after transplantation in NPM1 mutated AML: results from the UK NCRI AML17 study. Blood 135(9):680-688

Tsai P-C, Glastonbury CA, Eliot MN, Bollepalli S, Yet I, Castillo-Fernandez JE, Carnero-Montoro E, Hardiman T, Martin TC, Vickers A, Mangino M, Ward K, Pietilainen KH, Deloukas P, Spector TD, Vinuela A, Loucks EB, Ollikainen M, Kelsey KT, Small KS, Bell JT. 2018. Smoking induces coordinated

DNA methylation and gene expression changes in adipose tissue with consequences for metabolic health. *Clinical Epigenetics* 10:126.

## 15.1 Neutralizing antibody responses to SARS-CoV-2 arising from infection and vaccination

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**Project Description:**

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a betacoronavirus responsible for coronavirus disease-19 (COVID-19). Spike (S) is the virally encoded surface glycoprotein facilitating angiotensin converting enzyme-2 (ACE-2) receptor binding on target cells through its receptor binding domain (RBD). In a rapidly evolving field, researchers have already shown that, in most cases, individuals with a confirmed PCR diagnosis of SARS-CoV-2 infection develop IgM, IgA and IgG against the virally encoded surface spike protein (S) and nucleocapsid protein (N) within 1-2 weeks post onset of symptoms (POS) and remain elevated following initial viral clearance. S is the target for neutralizing antibodies (nAbs), and a number of highly potent monoclonal antibodies (mAbs) have been isolated that predominantly target the receptor binding domain. Recently, a new SARS-CoV-2 variant has been identified which contains ten S mutations across S1 and S2. This variant has been associated with a rapid increase in infection numbers within the UK and is now the dominant variant in London and the South East of England. It is not known whether these mutations will lead to escape from neutralizing antibodies generated in response to SARS-CoV-2 infection and/or vaccination.

This project will determine the sensitivity of the new virus variant to neutralizing monoclonal antibodies and sera/plasma from convalescent individuals to determine whether these mutations lead to antibody escape using a pseudovirus neutralization assay. Further, antigen-specific B cell sorting will be used to isolate S-specific neutralizing antibodies from COVID-19 vaccinated individuals to determine whether these antibodies target similar epitopes to those generated through natural infection. The student will learn a wide range of techniques including tissue culture, antibody cloning and expression, cell-based neutralization assays and ELISA.

**One representative publication from each co-supervisor:**

J. Seow, C. Graham, B. Merrick, S. Acors….. M.H. Malim, **K.J. Doores**,# Longitudinal observation and decline of neutralizing antibody responses in the three months following SARS-CoV-2 infection in humans, 2020, ***Nat Microbiol***, doi.org/10.1038/s41564-020-00813-8.

**2.** S. Pickering#, G. Betancor, R. P. Galão, B. Merrick#, ……, **K.J. Doores#** and J.D. Edgeworth, Comparative assessment of multiple COVID-19 serological technologies supports continued evaluation of point-of-care lateral flow assays in hospital and community healthcare settings, ***PLoS Pathog***, 2020, doi.org/10.1371/journal.ppat.1008817

## 16.1 The effect of lipid composition on mechanotransduction in health and disease.

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**Project Description:**

Mechanical forces regulate key biological functions, including growth and differentiation. When mechanobiology goes awry, it leads to dire pathologies, including muscular dystrophy, developmental defects, premature ageing and cancer. While most of our knowledge on mechanotransduction focuses on proteins, comparatively little is known about lipids.

*Are the lipids forming the plasma membrane and nuclear envelope (NE) dynamically modified under mechanical stress?* We will explore whether the lipidome changes in the plasma membrane and NE of cells exposed to mechanical stress. We will subject cultured cells to substrates of different stiffness, and extract their lipids, which will analysed by mass spectrometry. In parallel, we will use Atomic Force Microscopy (AFM) in combination with magnetic tweezers cell stretching experiments to probe the mechanical properties of membranes.

The student will gain expertise in single cell biophysics, combined with cell and molecular biology techniques. S/he will also gain deep knowledge in mass spectrometry. In Year 1, cell biology experiments will be performed at UE lab and the student will learn how to prepare substrates of different stiffness in SGM lab. Year 2 will be devoted to conduct single cell mechanical experiments using AFM and Magnetic Tweezers (SGM). During Year 3 the student will concentrate on lipidomics (UE). Experiments, analysis and paper writing will continue in Year 3-4.

This is a unique opportunity to explore fundamental biophysical questions of lipids during mechanotransduction at the single cell level, combining cutting-edge nanomechanical biophysical techniques (Garcia-Manyes) and modern cell biology and mass spectrometry (Eggert).

**<u>One</u> representative publication from <u>each co-supervisor</u>:**

Atilla-Gokcumen, Muro, E.; Relat-Goberna, J.; Sasse, S.; Bedigian, S.; Coughlin, M.L.; Garcia-Manyes, S.; Eggert, U.S.; «Dividing cells regulate their lipid composition and localization» *Cell* (2014), 156 (3), 428

Infante, E.; Stannard, A.; Board, S.J.; Rico-Lastres, P.; Rostkova, E.; Beedle, A.E.M.; Lezamiz, A.; Wang, Y.J.; Gulaidi Breen, S.; Panagaki, F.; Sundar Rajan, V.; Shanahan, C.; Roca-Cusachs, P.; Garcia-Manyes, S. The mechanical stability of proteins regulates their translocation rate into the cell nucleus. *Nature Physics*, 2019

## 17.1 An investigation into the mechanism of NAD modulation of beta cell mass in Type 2 Diabetes.

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**Project Description:**

Prevalence of type 2 diabetes (T2D) has increased dramatically in recent years. Reduced beta-cell functional mass is central to development of T2D. Elucidating the underlying disease mechanisms is crucial for devising novel strategies to prevent and treat T2D.

Previously we have shown that dysregulation of pancreatic islet beta-cell NAD levels, through altered function of the NAD-biosynthetic enzyme NAMPT, plays a key role in driving beta cell dysfunction in T2D. However, the precise mechanisms by which dysregulation of NAD metabolism leads to reduced functional beta-cell mass remain unclear.

*This project will examine whether NAD dysregulation drives beta-cell dysfunction via changes in epigenetic regulation of beta-cell gene expression.*

Aim 1 (Years 1-2): To examine the effects of NAD modulating compounds on beta-cell functional mass using mouse and human islets (e.g. insulin secretion radioimmunoassay, immunofluorescence assessment of beta-cell mass)

Aim 2 (Years 1-2): To use RNAseq and ATAC-seq methodology to generate genome-wide datasets from islet models and analyse these to identify effects of NAD modulation beta cell gene expression and chromatin accessibility.

Aim 3 (Year 3+): To analyse the data sets generated in Aim 2 by cross validating findings with other data resources and manipulating key targets identified to examine functional outcomes (informatics, gene knockdown, metabolism).

Together will provide a better understanding of how NAD modulating compounds modulate betacell functional mass, and identify additional targets for therapeutic intervention.

Skills training:
Cell culture, islet isolation, insulin secretion radioimmunoassay, luminescence apoptosis assays, BrdU proliferation assays; qRT-PCR, microscopy/immunofluorescence, high throughput RNA and ATAC sequencing, bioinformatics)

**<u>One</u> representative publication from <u>each co-supervisor</u>:**

Sayers SR, Beavil RL, Fine NHF, Huang GC, Choudhary P, Pacholarz KJ, Barran PE, Butterworth S, Mills CE, Cruickshank JK, Silvestre MP, Poppitt SD, McGill A, Lavery GG, Hodson DJ, Caton PW. Structurefunctional changes in eNAMPT at high concentrations mediate mouse and human beta-cell dysfunction in type 2 diabetes. Diabetologia, 2020 Feb;63(2):313-323

Holland ML, Lowe R, Caton PW, Gemma C, Carbajosa G, Danson AF,Carpenter AAM, Loche E, Ozanne SE, Rakyan VK. Early life nutrition modulates the epigenetic state of specific rDNA genetic variants in mice. *Science* 2016; 353: 495-8.

## 18.1 Base editing of inherited skin diseases for therapeutic application.

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**Project Description:**

The host lab has a track record around cell-based and *in vivo* gene therapies for patients with severe inherited skin diseases. Here, the opportunity is for the student to develop a new approach to therapy, gene editing – specifically the technique of base editing using specific nucleotide deaminases. Thus far we can: i) create induced pluripotent stem cells (iPSCs); ii) differentiate iPSCs into keratinocytes and fibroblasts; and, iii) genome edit using CRISPR-Cas9 systems. This new project will develop base editing in keratinocytes, fibroblasts or iPSCs focusing on the inherited blistering skin disease, dystrophic epidermolysis bullosa.

Specific Aim 1: Isolation of keratinocytes (KC) and fibroblasts (FB) from skin biopsies obtained from selected patients with inherited skin diseases, reprogramming primary KC or FB into iPSCs, and characterizing these cells (Year 1).

Specific Aim 2: Correction and verification of genetic mutations by the selected CRISPR-Cas-base gene editing method in KC, FB or iPSC (Year 2).

Specific Aim 3: Functional evaluation of the efficacy of gene-corrected patients' cells by testing the mRNA and protein expression levels, assessing the essential cell properties after gene correction, and generation of 3D skin constructs (Year 3).

Techniques and skills involved in this project: BE-Designer tool analysis on patient mutations; prediction analysis for "on-target" activity and potential "off-target" activity for each designed gRNA; CRISPR-Cas-base editing; cell culture; iPSC reprogramming and differentiation; Sanger Sequencing; high throughput DNA sequencing, protein biochemistry, western blotting, functional cellular assays, generation of 3D skin constructs.

**<u>One</u> representative publication from <u>each co-supervisor</u>:**

Jacków J et al. PNAS 2019;116: 26846-26852;

Lwin SM et al., JCI Insight 2019;4(11): e129243

## 19.1 The role of the actin cytoskeleton for cytotoxic T cells.

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**Project Description:**

Our group is interested in the understanding the signalling pathways that control the migration of cytotoxic T cells (CTLs) into tumour tissues and the subsequent killing of their target cells. We do this by a combination of *in vitro* biochemistry and cell biology approaches, including CRISPR knockouts, advanced microscopy techniques and *in vivo* mouse model approaches. Our lab has recently identified the kinase WNK1 as a novel and important regulator of T cell killing and actin dynamics. In the absence of WNK1, CTLs fail to migrate into tumours and do not properly rearrange their actin cytoskeleton upon binding to a target cell. As a consequence, they are strongly defective in killing.

In this project the student will use primary mouse T cells to study the role of WNK1 in controlling TCR induced actin remodelling. During the first year research will focus on imaging actin dynamics during T cell killing *in vitro* through advanced microscopy techniques to further characterize the phenotype. From the second year onwards, the student will use biochemistry approaches to investigate the molecular basis of WNK1-signalling, concentrating on pathways that control actin polymerization and acto-myosin contractility based on preliminary data from lab. This will be done through immuno-blotting experiments, CRISPR-knockouts and overexpression studies to identify WNK1-dependent pathways and from year three onwards through pull downs, interaction studies and phosphoproteomics approaches to identify how WNK1 activates those pathways.

The work will be carried out in the Köchl lab in collaboration with Andrew Cope's lab, a leading expert in studying T cell function.

**<u>One</u> representative publication from <u>each co-supervisor</u>:**

WNK1 kinase balances T cell adhesion versus migration in vivo. Köchl R, Thelen F, Vanes L, Brazão TF, Fountain K, Xie J, Huang CL, Lyck R, Stein JV, Tybulewicz VL. Nat Immunol. 2016 Sep;17(9):1075-83.

Burn GL, Cornish GH, Potrzebowska K, Samuelsson M, Griffie J, Minoughan S, Yates M, Ashdown G, Pernodet N, Morrison VL, Sanchez-Blanco C, Purvis H, Clarke F, Brownlie RJ, Vyse TJ, Zamoyska R, Owen DM, Svensson LM, Cope AP. Super-resolution imaging of the cytoplasmic phosphatase PTPN22 links integrin-mediated adhesion with autoimmunity. *Sci Signaling* 2016; 9: ra99.

## 20.1 Advanced live cell imaging based characterisation of a novel Scar/WAVE and Ena/VASP ligand in 3D pancreatic cancer cell invasion.

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**Project Description:**

Pancreatic ductal adenocarcinoma (PDAC) is a devastating cancer with very low survival rate due to the uncontrolled cell proliferation and metastatic spread. Metastasis is driven by changes in the regulation of the actin cytoskeleton, which provides the force for cell migration. Surprisingly little is known about the molecular details of how regulation of the actin cytoskeleton is changed to allow PDAC cells to become motile and how this defines the mode of invasion into 3D matrix from PDAC organoids. We have identified a novel regulator of the actin cytoskeleton and cell migration which binds to two important actin effectors, Ena/VASP and Scar/WAVE-Arp2/3 complex and which is phosphorylated by the proto-oncogenes, the tyrosine kinases c-Src and c-Abl. We also know that expression of this novel protein positively correlates with reduced survival of patients with PDAC.

In this project you will explore the function of this novel protein in invasion from PDAC spheroids and organoids, utilizing novel optical imaging strategies based on light-sheet fluorescence microscopy. The system will enable high-speed volumetric-imaging allowing quantification of invasion into the surrounding matrix in high detail. Combining FRET-FLIM capabilities of this state-ofthe-art microscope with image-based analysis tools written in MATLAB/Python will permit quantification of the activity of the Arp2/3 complex in the invading cells using a novel FRET-FLIM biosensor. You will use a combination of genetic (CRISPR-Cas9), biochemical and imaging approaches to explore regulation of the interactions of this novel protein with Ena/VASP and Scar/WAVE-Arp2/3 complex by Src and Abl and the functional significance of it.

**One representative publication from each co-supervisor:**

Law, A.-L., Jalal, S., Mosis, M., Pallett, T., Guni, A., Brayford, S., Yolland, L., Marcotti, S., Levitt. J.A., Poland, S.P., Rowe-Sampson, M., Jandke, A., Köchl, R., Pula, G., Ameer-Beg, S.M., Stramer, B.M., and Krause, M. (2020) Nance-Horan Syndrome-like 1 protein negatively regulates Scar/WAVEArp2/3 activity and inhibits lamellipodia stability and cell migration. Nature Communication, in revision; BioRxiv: https://www.biorxiv.org/content/10.1101/2020.05.11.083030v1

Poland, S P., Krstajić, N., Monypenny, J., Coelho, S., Tyndall, D., Walker, R. J., Devauges, V., Richardson, J., Dutton, N., Barber, P., Li, D-D, Suhling, K., Ng, T., Henderson, R. K. and Ameer-Beg, S. M., "A high speed multifocal multiphoton fluorescence lifetime imaging microscope for live-cell FRET imaging." Biomedical optics express 6, no. 2 (2015): 277-296.

## 21.1 Characterising the tumour-associated macrophage niche.

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**Project Description:**

Tumour-associated macrophages (TAM) are a critical component of the tumour-microenvironment and play important roles in cancer progression. However, we still understand little about the mechanisms that regulate TAM homeostasis and function. This project will focus on identifying and characterising the cellular components of the TAM niche in a mouse model of breast cancer (MMTVPyMT).

Year 1/2: Identifying TAM niches. a) Identify the cellular sources of CSF1 (the major growth/survival factor for macrophages) in PyMT tumours by flow cytometry and imaging using Csf1-reporter mice we have developed. b) Conditional deletion of Csf1 gene expression in candidate tumour-stromal cells using Cre/lox mediated gene-targeting.

Year 2/3: Interrogate TAM function via manipulation of their niche. We hypothesise that deletion of Csf1 in specific tumour stromal cells will ablate TAM. We will then assess the impact on tumourprogression and metastasis in the PyMT model.

Year 3/4: Characterizing tumour stroma-TAM dialogue. Single-cell RNA sequencing will be used to unravel the molecular basis of the stroma-TAM dialogue in PyMT tumours, through identification of pairs of ligands/receptors that are expressed by tumour stromal cells and TAM. The roles of the candidate molecules in the TAM niche will be tested using conditional gene targeting.

Both supervisors have extensive expertise in cancer biology. The Lawrence lab has specific expertise in TAM biology and has established genetic tools to study the cellular sources of Csf1 in intact tissues and the conditional deletion of Csf1 expression in specific cell lineages. The Ali lab has extensive experience with studying tissue-resident immunity and associated experimental models.

**<u>One</u> representative publication from <u>each co-supervisor</u>:**

Goossens P, Rodriguez-Vita J, Etzerodt A, …Lawrence T. Membrane Cholesterol Efflux Drives TumorAssociated Macrophage Reprogramming and Tumor Progression. *Cell Metab*. 2019;4131(19):30128-
7.

Ali N et al., Cell . 2017 Jun 1;169(6):1119-1129.e11. doi: 10.1016/j.cell.2017.05.002.

## 22.1 The NoCut checkpoint-regulation of membrane remodelling during cell division and basis for cancer association.

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**Project Description:**

Cell division is highly coordinated to ensure the faithful segregation of the duplicated genome between the daughter cells. Cytokinetic abscission finishes cell division by severing the midbody, a membranous bridge that connects the daughter cells. Membrane severing in abscission is facilitated by the endosomal sorting complex required for transport (ESCRT) machinery. The polymerization of ESCRT-III, a complex that serves as membrane "scissors", mediates abscission by drawing together the opposing membranes at the midbody. Importantly, abscission is tightly regulated by the NoCut checkpoint, which delays midbody resolution in response to mitotic conflicts. We have identified CHMP4C as a NoCut component that regulates ESCRT-III. Critically, a human polymorphism in CHMP4C (CHMP4C$^{T232}$) increases susceptibility to cancer in 7% of the UK population. We have explained this cancer association by showing the loss of NoCut activity and genetic instability in cells expressing CHMP4C$^{T232}$.

This project will combine cell biology, molecular biology, advanced microscopy and biophysics to explore how NoCut regulates membrane remodelling at the midbody. Specifically, the student will learn quantitative live-cell microscopy to explore ESCRT-III dynamics during abscission, and how the cancer-associated allele of CHMP4C regulates ESCRT-III activity at the midbody. Novel biophysical approaches will be developed to determine whether NoCut regulates the polymeric estate of ESCRTIII. Super-resolution microscopy will complement these studies by determining the nanoscale map of NoCut-arrested midbodies. In summary, this project will uncover the molecular basis of novel oncogenic mechanisms, and how these may contribute to cancer in individuals that carry NoCutinactivating alleles.

**<u>One</u> representative publication from <u>each co-supervisor</u>:**

A cancer-associated polymorphism in ESCRT-III disrupts the abscission checkpoint and promotes genome instability. Sadler JBA, Wenzel DM, Williams LK, Guindo-Martínez M, Alam SL, Mercader JM, Torrents D, Ullman KS, Sundquist WI, Martin-Serrano J. Proc Natl Acad Sci U S A. 2018 Sep 18;115(38):E8900-E8908. doi: 10.1073/pnas.1805504115.

Single-cell glycolytic activity regulates membrane tension and HIV-1 fusion. Coomer CA, CarlonAndres I, Iliopoulou M, Dustin ML, Compeer EB, Compton AA, Padilla-Parra S. PLoS Pathog. 2020 Feb 21;16(2):e1008359. doi: 10.1371/journal.ppat.1008359. eCollection 2020

## 23.1 Immune cell invasion in sepsis and Covid-19: how can we block it?

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**Project Description:**

In past work we have shown that hydrogen peroxide is a potent chemoattractant that guides immune cells towards invading pathogens, thus enabling these threats to be neutralised. However, when the body is overwhelmed by pathogens, as happens in serious cases of bacterial sepsis, seasonal flu or Covid-19, high levels of $H_2O_2$ trigger the release of cytokines from immune cells, causing a "cytokine storm", and promote the release of neutrophil extracellular traps (NETs), sticky secretions of DNA that are beneficial in trapping bacteria, but cause damage in high concentrations. How can these adverse events be prevented?

Our lab has also shown that the effects of $H_2O_2$ on immune cells are mediated by TRPM2, a calciumpermeable membrane ion channel that floods immune cells with calcium when $H_2O_2$ is high. Can we inhibit cytokine storms and NET release by blocking TRPM2? We will measure calcium influx, neutrophil movement, cytokine and NET release both *in vitro* and *in vivo*. We will explore TRPM2 blockers that may have therapeutic use in life-threatening diseases such as sepsis and Covid-19. We anticipate that the lab project will tie up with a clinical trial, run by Dr Shankar-Hari, of one promising TRPM2 blocker.

Techniques: imaging immune cell movement and intracellular calcium in vitro and in vivo; measurement of cytokine and NET release in vitro and in vivo; patch clamp electrophysiology; molecular biology.

Objectives:
Year 1 – dependence of cytokine and NET release on TRPM2 activation in vitro.
Year 2 – immune cell motility, cytokine and NET release in vivo.
Years 3, 4 – realistic models of systemic sepsis and acute respiratory distress syndrome (a sepsis-like syndrome in the lungs) triggered by viral and bacterial antigens in animal models. Therapeutic value of TRPM2 blockers. Tie-up with clinical trials.

**One representative publication from each co-supervisor:**

Tan CH, McNaughton PA. (2016) The TRPM2 ion channel is required for sensitivity to warmth. Nature. 536:460-3.

Robbins J, Passmore GM, Abogadie FC, Reilly JM & Brown DA (2013) Effects of KCNQ2 gene truncation on M-type Kv7 potassium currents. PLoS ONE 8(8): e71809. doi:10.1371/journal.pone. 0071809

## 24 Novel multi-disciplinary strategy to identify and target unconventional (neo)epitopes suitable for immunotherapy against colorectal cancer.

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**Project Description:**

Immunotherapies are at the cutting-edge of the translational research against cancer and neoepitopes carrying tumour-specific mutations are ideal targets. Proteasome-generated spliced epitopes are a promising - although almost uncharted - source of targets for immunotherapy against cancer.

We hypothesize that spliced neoepitopes can be targeted by CD8+ T cells upon immune checkpoint inhibitors and adoptive T cell therapies against colorectal cancer.
The objective of this project is identifying spliced neoepitopes carrying tumour-specific mutations in colorectal cancer samples and studying their involvement in immunotherapy-induced immune response.

In particular, the PhD student will:

- develop a predictor of non-spliced and spliced neoepitopes and validate it through biochemical experiments;
- investigate the presence of spliced neoepitopes in association with mutation burden of a cohort of colorectal cancers;
- study the association between predicted neoepitopes, mutation clonality and response to immune checkpoint inhibitor;
- study tumour infiltrating CD8+ T cell clones identified in a cohort of colorectal cancers and study their potential recognition of non-spliced and spliced neoepitopes;
- isolate and clone TCRs specific for clonal and recurrent non-spliced and spliced neoepitopes;
- study the efficacy of neoepitope-specific TCR-transduced T cells in killing cancer cells in a colorectal organoid system.

The project will be carried out at Francis Crick Institute in Mishto lab and Ciccarelli lab.

The project might generate patentable material and will benefit from international collaborations (e.g. Max Planck Institute).

The project is multi-disciplinary and will combine genetics & genomics, system biology, bioinformatics, biochemistry, molecular & cellular immunology applied to cancer.

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**<u>One</u> representative publication from <u>each co-supervisor</u>:**

Liepe J, Marino F, Sidney J, Jeko A, Bunting DE, Sette A, Kloetzel PM, Stumpf MP, Heck AJ, Mishto M. A large fraction of HLA class I ligands are proteasome-generated spliced peptides. *Science 2016* Oct; 354(6310): 354-358. DOI: 10.1126/science.aaf4384.

Ciccarelli FD. Mutation differ in normal and cancer cells of the oesophagus. *Nature 2019* Jan; 565(7739):301-303

## 25 Identifying genetic determinants of Antimicrobial Resistance in the microbiome using Functional Metagenomics.

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**Project Description:**

Antimicrobial drug resistance (AMR) is a serious and escalating threat to human health of global concern. The WHO has highlighted surveillance of AMR as a priority area. Despite continued research, our knowledge of genetic determinants driving antimicrobial resistance within microbial communities *in vivo* is incomplete. Access to microbial genome sequences is an important first step in identifying genes contributing to AMR. However, knowledge of genome sequence alone does not tell us whether these genes are *functionally active* in a microbial community. This ambitious research project will combine microbiology, molecular biology, functional metagenomics, nextgeneration sequencing and bioinformatics to address this knowledge gap.

Microbial DNA extracted from human saliva and clinically relevant fungal species will be used to create metagenomic DNA libraries which will be pooled and introduced into clinically relevant species of oral bacteria and fungi. Recipient microbes will be cultured in the presence of different antibiotics. Surviving colonies demonstrating resistance will be enriched for functionally active AMR genes (ARGs). The libraries will be extracted from these colonies and characterised using next-generation sequencing (Illumina and Oxford Nanopore technologies), and a combination of bioinformatics including CARD and MARDy databases to identify ARGs, and other tools to identify associated gene sequences. This research will significantly enhance our understanding of AMR in microbial communities, with major implications for its surveillance.

Over-arching objectives:

i) Development of functional metagenomic tools for oral bacteria and fungi.
ii)Identification and characterisation of both new and known ARGs. iii) Functional analysis of genetic elements involved in driving AMR.

**One representative publication from each co-supervisor:**

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Carr, V.R.; Witherden, E., Lee, S., Shoaie, S., Mullany, P., Proctor, G.B., Gomez-Cabrero, D., Moyes, D.L. Abundance and diversity of resistomes differ between healthy human oral cavities and gut (2020) *Nature Communications* 11,693

Richardson JP, Mogavero S, Moyes DL, Blagojevic M, Krüger T, Verma AH, Coleman BM, De La Cruz Diaz J, Schulz D, Ponde NO, Carrano G, Kniemeyer O, Wilson D, Bader O, Enoiu SI, Ho J, Kichik N, 26

Role of MKP1 in fungal and bacterial disease.

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**Project Description:**

MKP1 is a phosphatase that regulates host responses via the MAPK (mitogen-activated protein kinase) pathway. *In vitro*, MKP1 is activated via surface receptors and cell damage caused during infection, and is critical in mediating immune responses against both fungal (*Candida albicans* – causative agent of thrush) and bacterial pathogens associated with periodontitis. Currently, the role of MKP1 *in vivo* during these fungal and bacterial diseases is unknown. This project aims to determine the role of MKP1 in *Candida* infections and periodontitis *in vivo.*

Healthy and MKP1 knockout mice (available at King's College London) will be infected with *Candida* pathogens mucosally and systemically, or orally with the periodontal pathogen, *Porphyromonas gingivalis,* using the gavage model. Infection over time will be monitored by assessing microbial burden and population structure, tissue invasion, periodontal bone destruction, weight loss and other standard clinical outcomes/observations. The innate and adaptive immune response (cellular, humoral) will be assessed to determine the importance of MKP1 in protecting against or affording susceptibility to these fungal and bacterial infections.
Molecular (RNASeq, qPCR) and cellular (flow cytometry, Luminex) analysis of host tissues will help determine protective or susceptible immune profiles. Inhibition of MKP1 or immune molecules (identified during molecular/cellular analysis) will determine the therapeutic potential of targeting MKP1-associated immune factors during disease.

This multidisciplinary project will combine infection, immunity, microbiology, molecular and cellular analyses to reveal the role of MKP1 during fungal and bacterial diseases, define new mechanisms in host-microbial responses, and may identify MKP1 as a novel therapeutic target during infection.

**<u>One</u> representative publication from <u>each co-supervisor</u>:**

Moyes DL, Wilson D, Richardson JP, Tang SX, Wernecke J, Höfs S, Gratacap RL, Mogavero S, Robbins J, Runglall M, Murciano C, Blagojevic M, Thavaraj S, Förster TM, Hebecker B, Kasper L, Vizcay G, Iancu SI, Kichik N, Häder A, Kurzai O, Cota E, Bader O, Wheeler RT, Gutsmann T, Hube B and Naglik JR (2016). Candidalysin is a fungal peptide toxin critical for mucosal infection. *Nature* 532, 64-68.

Xu Q, Shoji M, Shibata S, Naito M, Sato K, Elsliger MA, Grant JC, Axelrod HL, Chiu HJ, Farr CL, Jaroszewski L, Knuth MW, Deacon AM, Godzik A, Lesley SA, Curtis MA, Nakayama K, Wilson IA. (2016). A Distinct Type of Pilus from the Human Microbiome. *Cell*, 165(3):690-703.

## 27 Antiviral targeting of influenza virus and Ebola virus ribonucleoprotein complexes by TRIM25.

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**Project Description:**

TRIM25, an E3 ubiquitin ligase, plays a key role in innate immunity as part of viral RNA sensing pathways and by regulating zinc-finger antiviral protein (ZAP) activity. ZAP is an RNA-binding protein that inhibits a wide range of viruses by targeting viral and cellular RNAs, particularly those enriched in CpG dinucleotides. How TRIM25 controls viral RNA sensing and ZAP antiviral activity remains unknown, but recently TRIM25 was shown to associate with influenza A virus (IAV) ribonucleoprotein (vRNP) complexes. Additionally, we have data suggesting that TRIM25 interacts with the vRNP of Ebola virus (EBOV), dissociating the viral nucleoprotein (NP) from the genomic RNA and exposing it to ZAP-mediated restriction. Sensitizing RNA viruses to ZAP and other RNA-directed antiviral mechanisms is a promising strategy that we are developing to create new live attenuated vaccines.

The student will use molecular virology, biochemistry, transcriptomics and real-time fluorescent microscopy to understand how the interaction between TRIM25 and the vRNP of IAV and EBOV inhibits viral replication.

Aim 1: Characterize the molecular determinants of species-specific TRIM25 interactions with IAV and EBOV vRNPs, their effect on viral replication, and their role in recruiting ZAP.

Aim 2: Use live imaging microscopy to understand the subcellular localization of TRIM25 and ZAP recruitment to vRNPs, and the relationship with viral RNA sensing pathways.

Aim 3: Use RNA-seq to determine how TRIM25 regulates gene expression in response to IAV infection.

Aim 4: Investigate the role of RNA-binding and ubiquitination in TRIM25-mediated restriction of IAV and its counteraction by IAV NS1.

**One representative publication from each co-supervisor:**

Ficarelli M, Antzin-Anduetza I, Hugh-White R, Firth AE, Sertkaya H, Wilson H, Neil SJD, Schulz R, Swanson CM. (2020) CpG Dinucleotides Inhibit HIV-1 Replication through Zinc Finger Antiviral Protein (ZAP)-Dependent and -Independent Mechanisms. *J Virol.* 94:e01337-19.

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Ficarelli M, Wilson H, Pedro Galão R, Mazzon M, Antzin-Anduetza I, Marsh M, Neil SJ, Swanson CM. (2019) KHNYN is essential for the zinc finger antiviral protein (ZAP) to restrict HIV-1 containing clustered CpG dinucleotides. *Elife*. 8:e46767.

## 28 Does SARS-CoV-2 target Calcium/Calmodulin signalling to cause disease?

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**Project Description:**

Calmodulin (CaM) is the main mediator of $Ca^{2+}$ signalling and controls a wide range of cellular processes in eukaryotic cells including immune signalling. $Ca^{2+}$ homeostasis is exploited by several viruses such as SARS-CoV-1 to promote infection and dysregulate host immune responses. Whether the causative agent of the current COVID-19 pandemic SARS-CoV-2 also manipulates $Ca^{2+}$/CaM pathways has not yet been studied. Since many virulence factors are shared between the two CoV strains, **we hypothesise that SARS-CoV-2 targets $Ca^{2+}$/CaM signalling to cause disease.**

The aims of this project are to:

1. Identify putative SARS-CoV-2 viral antagonists of CaM-mediated-signalling (Year 1) and define the molecular mechanisms responsible (Year 2-3)
2. Determine the CaM-dependent signalling pathways targeted by these factors (Year 1-2)
3. Characterise the potential antiviral roles of CaM-dependent immune-pathways in the replication of SARS-CoV-2 (Year 2-3)

We will first determine if SARS-CoV-2 virulence factors directly bind CaM and prevent activation of normal CaM targets like CAM kinases. To identify which CaM-dependent pathways may be targeted by viral virulence factors, we will use reporter assays. We will then use a reverse genetics-based system that will allow specific SARS-CoV-2 proteins to be mutated and evaluate the impact on viral infection. Finally, we will investigate the potential roles of CaM signalling in promoting or preventing viral infection.

Techniques used will include:

- Molecular Biology and Virology
- Biochemistry
- Tissue culture / CRISPR-Cas9-mediated gene editing
- Flow cytometry and microscopy
- Full-length virus work in BSL3 containment lab
- Reverse-genetics viral systems

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**<u>One</u> representative publication from <u>each co-supervisor</u>:** Odendall, C., Voak, A., Kagan, J. (2017). Type III IFNs Are Commonly Induced by Bacteria-Sensing TLRs and Reinforce Epithelial Barriers during Infection. *The Journal of Immunology* 199(9), 3270 - 3279.

Galão, R.P., Pickering, S., Neil S.J.D., et al (2014). Retroviral retention activates a Sykdependent HemITAM in human tetherin. *Cell Host and Microbe* 16, 3, 291-303.

## 29 Understanding chromatin remodelling at the nuclear periphery.

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**Project Description:**

Eukaryotic genomes are highly organized within membrane-bound nuclei. Most cell types tether heterochromatin – a complex set of transcriptionally repressed chromatin domains – to a protein meshwork occupying the inner nuclear membrane (INM). This tethering establishes proper spatial organization of chromosomes within the nucleus and can promote silencing of tethered chromatin, allowing high-level regulation of gene expression. Mutations in INM heterochromatin-interacting proteins (e.g., MAN1, LBR) can manifest in human diseases known as nuclear envelopathies, and heterochromatin dysfunction can increase cancer susceptibility. In spite of profound fundamental and clinical interest, we know little about the molecular mechanisms underlying this sub-nuclear chromatin domain organisation and the extent of its functional significance. Importantly, we do not understand how patterns of chromatin organisation can be maintained throughout many cell divisions, or how cells reorganize their chromatin-NE interactions during cell fate change. Working in mammalian and yeast cell biology labs, you will answer these questions by building on our observations in fission yeast, indicating that the membrane remodeller ESCRT-III/Vps4 may promote the dynamic turnover of heterochromatin-NE attachments, through its interactions with the evolutionarily conserved INM protein Lem2.

Y1. Developing tools to assess chromatin dynamics at the NE and to allow acute manipulation of ESCRT-III/Vps4 function in human cells (*molecular biology, biochemistry*).

Y2. Probing the roles of INM proteins (including Lem2) and ESCRT-III/Vps4 in chromatin restructuring at the nuclear periphery during cell division and differentiation (*RNAi, CRISPR, advanced microscopy*).

Y3-Y4. Obtaining mechanistic insights into the observed phenotypes (*stable/knock-in cell line generation, yeast genetics, cell fate studies*).

**One representative publication from each co-supervisor:**

Pieper, G., Sprenger, S., Teis, D. and S. Oliferenko. 2020. ESCRT-III/Vps4 controls heterochromatinnuclear envelope attachments. *Developmental Cell* 53:27-41

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Olmos Y, Hodgson L, Mantell J, Verkade P and Carlton JG. 2015. ESCRT-III controls nuclear envelope reformation. *Nature.* 522:236-239.

## 30.1 Regulation of cancer cell signalling by B7-H3.

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**Project Description:**

B7-H3 (CD276) is a transmembrane receptor and one of seven B7 superfamily immune checkpoint molecules involved in regulating tumour-immune cell interactions. B7-H3 is highly overexpressed in a number of cancer types including lung, breast, colon, renal, and ovarian. B7-H3 is an appealing target for the development of therapeutic agents and this has resulted in the development of humanized anti-B7-H3 antibodies that delay the growth of primary tumors in preclinical studies and are now in clinical trials. However, the physiological ligand and functions of B7-H3 in cancer cells remain unknown.

Recent unpublished work in the Parsons lab has shown that B7-H3 localises to human lung carcinoma cell-cell junctions and contributes to cell-cell adhesion, actin organisation and invasion. We have further shown that B7-H3 localises to cilia in normal lung epithelial cells, and that overexpression of B7-H3 leads to loss of cilia formation. The goal of this project is to use a range of molecular, biochemical and state-of-the-art microscopy approaches define the extracellular cues and signaling contributions from B7-H3 that regulate these functions in human lung cancer cells. The key aims are:

1. Define ligands for B7-H3 and map domain requirements for cilia and junction localisation (Yr1)
2. Test functional roles for B7-H3 ligands in regulating localization, signalling and cancer cell behaviour (Yrs2/3)
3. Analyse B7-H3 localisation in human lung tumours and correlations with ligand/signalling targets (Yrs2/3)
4. Inhibit B7-H3 cilia/signalling function and determine outcomes to tumour cell behaviour (Yr4)

Data arising from this study will shed light on the novel physiological functions of B7-H3 and may provide new routes to therapeutic intervention.

**<u>One</u> representative publication from <u>each co-supervisor</u>:**

KIF22 coordinates CAR and EGFR dynamics to promote cancer cell proliferation. Pike, R., OrtizZapater, E., Lumicisi, B., Santis, G. & Parsons, M., 30 Jan 2018, Science Signaling. 11, 515, eaaq1060.

The architecture of EGFR's basal complexes reveals autoinhibition mechanisms in dimers and oligomers. Zanetti-Domingues, L. C., Korovesis, D., Needham, S. R., Tynan, C. J., Sagawa, S., Roberts, S. K., Kuzmanic, A., Ortiz-Zapater, E., Jain, P., Roovers, R. C., Lajevardipour, A., van Bergen en Henegouwen, P. M. P., Santis, G., Clayton, A. H. A., Clarke, D. T., Gervasio, F. L., Shan, Y., Shaw, D. E., Rolfe, D. J., Parker, P. J. Martin-Fernandez M, , 1 Dec 2018, Nature Communications. 9, 1, 4325.

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## 31 From coronavirusto hyperinflammation: deciphering the role of lectin recognition in complement pathway activation and inflammation.

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**Project Description:**

One of the first points of contact of pathogenic coronaviruses during infection is the alveolar epithelium of the lung. A consequence of infection of these cells is a dysregulated inflammatory response typified by hyper-production of inflammatory mediators that leads to an acute respiratory distress syndrome (ARDS) in severe cases of SARS-CoV-2. We believe that this 'cytokine storm' is initiated through the SARS-CoV-2 activation of the complement-lectin pathway (a signalling cascade that plays a crucial role in pathogen sensing and clearance and as a major inducer of other mediators of inflammation). However, our understanding of how the virus drives the initiation and maintenance of this abnormal response is currently incomplete.

This project will use molecular and biochemical techniques together with cell-based models and imaging techniques to investigate the mechanisms and molecular interactions that underlie the response of alveolar epithelial cells to SARS-CoV-2. Findings from this project has the potential to lead new therapeutic approaches to coronavirus and other emerging pandemic viruses.

Year 1: will establish in vitro models of SARS-COV-2 infection of alveolar epithelial cells using both pseudovirus reporter systems and live wild type virus.

Year 2: will determine the molecular basis for the activation of the lectin complement pathway by SARS-CoV-2 structural proteins using biochemical, molecular and pharmacological approaches.

Year 3: will use in vitro co-culture with monocyte/ macrophages to determine how complement activation in alveolar epithelial cells leads to the hyperinflammatory response.

This exciting project interfaces host-pathogen biology, complement biology and immunology and will encourage student growth and development in a supportive research environment.

**<u>One</u> representative publication from <u>each co-supervisor</u>:**

C Farrar, D Tran, K Li, W Wu, Q. Peng, W Schwaeble, W Zhou, S Sacks. Collectin-11 detects stressinduced L-fucose pattern to trigger renal epithelial injury. Journal of Clinical Investigation2016 2;126(5):1911-25.
Polycarpou A, Howard M, Farrar CA, Greenlaw R, Fanelli G, Wallis R, Klavinskis LS,Sacks S

(2020)Rationale for targeting complement in COVID-19. EMBO Mol Med. 2020 Aug 7;12(8): e12642. doi: 10.15252/emmm.202012642

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## 32 Deciphering the amplicons on 1q that drive breast cancer and their association with different breast cancer subtypes.

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**Project Description:**

Additional copies of the 1q chromosome are an early event in breast tumorigenesis and are common in pre-cancerous lesions such as ductal carcinoma *in situ* (DCIS). It is not clear how 1q gain leads to a growth advantage, but it is possible that it allows aberrant cells to avoid immune surveillance, and this hypothesis will be investigated during the PhD. In invasive breast cancer is it common to see smaller regions of 1q amplification on the background of this initial whole arm gain however these copy number changes are not consistent across the different breast cancer subtypes.

The aim of this project is to ascertain (i) why certain regions of 1q are amplified in breast cancer; (ii) whether the genes within these chromosomal regions are key initiators of oncogenic pathways at different stages of cancer evolution; (iii) how the immune cells in the tumour microenvironment (TME) influence tumorigenesis. The ultimate translational aim is the identification of new therapeutic targets.

The project will have a strong bioinformatic focus but will also involve wet lab techniques.

Year 1: analyses of copy number, RNA seq and methylation data that has already been generated from a large cohort of DCIS and triple negative breast cancer

Year 2: generation and analyses of the above from other subtypes of breast cancer; mapping the spatial composition of immune cells in the TME

Year 3: model the evolution of cells with 1q gain and immune cells of the TME, functional analyses in cancer lines of genes identified above.

**<u>One</u> representative publication from <u>each co-supervisor</u>:**

Frequency of pathogenic germline variants in *BRCA1, BRCA2, PALB2, CHEK2* and *TP53* in ductal carcinoma in situ diagnosed in women under the age of 50 years. Petridis C, Arora I, Shah V, Megalios A, Moss C, Mera A, Clifford A, Gillett C, Pinder SE, Tomlinson I, Roylance R, Simpson MA, Sawyer EJ. *Breast Cancer Res. 2019 May 6;21(1):58.*

Integrated genomics and functional validation identifies malignant cell specific dependencies in Triple Negative Breast Cancer. Patel N, Weekes D, Drosopoulos K, Gazinska P, Noel E, Rashid M, Mirza H, Quist J, Brasó-Maristany F, Mathew S, Ferro R, Mendes Pereira A, Prince C, Noor A, Francesch-Domenech E, Marlow R, de Rinaldis E, Grigoriadis A, Linardopoulos S, Marra P, Tutt ANJ. *Nat Commun. 2018 Mar 13;9(1):1044. doi: 10.1038/s41467-018-03283-z. PMID: 29535384*

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## 33 Elucidating the mechanisms underlying lineage reprogramming for future diabetes therapies.

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**Project Description:**

Differentiated cells can be reprogrammed to switch identities from one cell type to another under the direction of specific transcription factors. Discovering the mechanisms underpinning lineage reprogramming will lead to improved cell fate engineering strategies and have profound implications for regenerative medicine. *The aim of this project is to generate a roadmap for lineage reprogramming of functional pancreatic* β*-cells from hepatocytes with the ultimate goal of curing diabetes.* My laboratory previously established a strategy to reprogram liver cells into pancreatic progenitors based on a single transcriptional regulator (Cerda-Esteban et al. 2017). *More recently, we have used single-cell* (*sc)-RNASeq to reconstruct the reprogramming path of human hepatocytes into pancreatic cells and characterize cellular heterogeneity during cell fate conversion processes.*

During the rotation project, the student will learn lineage reprogramming approaches and scRNASeq analysis.

Aim 1 (Years 1/2): To use sc-cell RNASeq to define mechanisms that can improve the efficiency and the outcome of reprogramming approaches to convert hepatocytes into functional pancreatic βcells.

Aim 2 (Years 2/3): Next, the candidate will study the identified reprogramming mechanisms and putative reprogramming barriers. CRISPR/CAS9 genome-editing technologies will be used to functionally test the identified candidate factors.

Final year: Molecular and functional properties of the reprogrammed cells, obtained from hepatocytes, will be benchmarked against endogenous β-cells.

The PhD student will acquire cutting-edge techniques in cell culture, confocal microscopy, transcriptome and Crispr/Cas9 technology established in the Spagnoli lab and bioinformatics skills in the Vigilante lab.

**<u>One</u> representative publication from <u>each co-supervisor</u>:**

Cozzitorto C, Mueller L, Ruzittu S, Mah N, Willnow D, Darrigrand JF, Wilson H, Khosravinia D, Mahmoud AA, Risolino M, Selleri L, Spagnoli FM. A Specialized Niche in the Pancreatic Microenvironment Promotes Endocrine Differentiation. *Dev Cell*. 2020 Aug 20:S15345807(20)30627-4. doi: 10.1016/j.devcel.2020.08.003. Online ahead of print.

Vigilante A, Laddach A, Moens N, Meleckyte R, Leha A, Ghahramani A, Culley OJ, Kathuria A, Hurling C, Vickers A, Wiseman E, Tewary M, Zandstra PW; HipSci Consortium, Durbin R, Fraternali F, Stegle O, Birney E, Luscombe NM, Danovi D, Watt FM. Identifying Extrinsic versus Intrinsic Drivers of Variation in Cell Behavior in Human iPSC Lines from Healthy Donors. *Cell Rep.* 2019 Feb 19;26(8): 2078-2087.e3. doi: 10.1016/j.celrep.2019.01.094.

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## 34 Inner ear organoids to model human ear development and disease.

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**Project Description:**

Hearing loss ranks among the top 10 health burdens worldwide and is the most common sensory disorder. However, as a 'hidden' disability is often overlooked, yet impacts on many aspects of daily life from communication with each other to appreciation of music or orientation in space. In the UK, 1 in every 650 babies is born with some form of hearing loss with more than 75% due to genetic mutations, of which about one quarter remain to be identified. This prevents early diagnosis, developing treatment plans and new therapeutic approaches.

While much progress has been made in identifying the molecular mechanisms that control ear development in animal models, our understanding of human ear formation is in its infancy. This project will address this gap by modelling human ear development in an iPSC-derived organoid system.

In the first year, the student will generate reporter lines for ear progenitors, neurons and hair cells in human iPSCs using Crispr/Cas9 and learn to establish ear organoids (collaboration with K. Koehler, Harvard). In year 2, s/he will characterise normal ear morphogenesis and cell type specification using transcriptome and epigenomic analysis. Finally, the student will study how mutations identified in congenital deafness patients lead to ear malformations using organoids as a model.

The project will advance our knowledge of human ear formation, define the molecular and cellular mechanisms involved and provide a new model to analyse disease phenotypes. In the long term, this system will not only be useful for modelling human disease, but also for drug screening and testing new therapeutic approaches.

**One representative publication from each co-supervisor:**

Chen, J., Tambalo, M., Barembaum, M., Ranganathan, R., Simoes-Costa, M., Bronner, M. E. & Streit, A., 2017. A systems level approach reveals new gene regulatory modules in the developing ear. Development 144, 8, p. 1531-1543

Hagemann, Cathleen; Tyzack, Giulia; Taha, Doaa; Devine, Helen; Greensmith, Linda; Newcombe, Jia; Patani, Rickie~; Serio, Andrea~; Luisier, Raphaëlle~ Automated and unbiased classification of motor neuron phenotypes with single cell resolution in ALS tissue. DOI10.1101/2020.08.17.253773

## 35.1 Immunological cross-talk: deciphering how human CD4+ T cells alter myeloid cell function.

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**Project Description:**

*Background*: Myeloid cells (monocytes, macrophages and dendritic cells) are key in the initiation, propagation and resolution of inflammation, in part through interactions with CD4+ T-cells. Whilst polarisation of T-cells by myeloid cells has been studied extensively, myeloid cells themselves can also be modulated upon interaction with effector (Teff) and regulatory (Treg) CD4+ T cells. However, the underlying mechanisms and functional consequences of CD4+ Tcell-mediated modulation of myeloid cells require further elucidation.

Our previous work demonstrated that *in vitro* co-culture of monocytes with Tregs polarises monocytes to a less inflammatory phenotype (Tiemessen PNAS 2007). In contrast, upon co-culture with effector T-cells, monocytes exhibit a more activated, pro-inflammatory phenotype (Tiemessen PNAS 2007; Jagger J Autoimmunity 2012). Furthermore, our recent unpublished RNA-sequencing data reveal differentially expressed genes and pathways in Teff-modulated monocytes and Tregmodulated monocytes, vs. monocytes cultured alone.

*Overarching aim and techniques:* to functionally evaluate the gene signatures of Teff-modulated vs. Treg-modulated monocytes in terms of myeloid cell function. Techniques will include human peripheral blood cell isolation and culture, iPSC-derived macrophage culture, multiparameter flow cytometry, cytokine detection (ELISA, Luminex, qRT-PCR), RNA-sequencing, gene knockdown and overexpression studies, epigenetic modification analysis, bioinformatic analysis.

*Specific objectives and timelines:*

- Objective 1: Get acquainted with relevant techniques and preliminary data, and select and validate candidate genes/pathways for follow up research based on literature searches, biological or disease relevance (year 1).
- Objective 2: Determine the biological relevance of candidate genes/pathways that are differentially expressed in myeloid cells upon interaction with effector vs regulatory Tcells using primary monocytes and iPSC-derived macrophages (year 1/2).
- Objective 3: Investigate whether myeloid cells undergo epigenetic modification upon (repeated) interaction with effector vs. regulatory T-cells (year 2/3 or 3/4).

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**<u>One</u> representative publication from <u>each co-supervisor</u>:**

IKZF3/Aiolos Is Associated with but Not Sufficient for the Expression of IL-10 by $CD4^+$ T Cells. Ridley ML, Fleskens V, Roberts CA, Lalnunhlimi S, Alnesf A, O'Byrne AM, Steel KJA, Povoleri GAM, Sumner J, Lavender P, Taams LS. J Immunol. 2020 Jun 1;204(11):2940-2948. doi: 10.4049/jimmunol.1901283.

Loss of IL-10 signaling in macrophages limits bacterial killing driven by prostaglandin E2. Mukhopadhyay S, Heinz E, Porreca I, Alasoo K, Yeung A, Yang HT, Schwerd T, Forbester JL, Hale C, Agu CA, Choi YH, Rodrigues J, Capitani M, Jostins-Dean L, Thomas DC, Travis S, Gaffney D, Skarnes WC, Thomson N, Uhlig HH, Dougan G, Powrie F. Journal of Experimental Medicine, 2020 Feb 3;217(2) [PMID: 31819956] DOI: 10.1084/jem.20180649

## 36.1 Co-ordinating hearing: Mechanisms underlying congenital birth defects associated with the ear.

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**Project Description:**

During development organs are formed from the integration of different tissues to produce a final complex structure. Often a single functional organ has a number of distinct embryonic origins, with components that initiate in different areas of the embryo at different times. This is particularly true of the ear, which is divided into the external, middle and inner ear. Signalling is essential to link up the ear into a chain for the transfer of sound. Defects in this process lead to conductive hearing loss, where sound fails to reach the inner ear (1 in 10,000 births).

Knowledge of the processes involved during ear development is important not only for understanding the underlying causes of congenital hearing loss, but also to provide the principles to recreate structures. It can, therefore, inform our understanding of how to reconstruct an effective ear canal in children where the canal is missing.

This proposal aims to address how the ear is integrated during development to create a functional organ. The project combines developmental biology with analysis of patient scans.

Aim 1: To understand the signals that integrate the different parts of the ear during development
Aim 2: To use patient data sets to assess how defects in one part of the ear impact other parts.
Aim 3: To provide mechanisms to explain congenital ear defects

Skills training: The student will be trained in molecular biology techniques, developmental biology and imaging, and immersed in clinically relevant problems. Critical thinking, presentation and writing skills will be taught.

**One representative publication from each co-supervisor:**

Thompson, H. Tucker, A.S. (2013). Dual origin of the epithelium of the middle ear. Science 339, 14531456.

Pai I, Crossley E, Lancer H, Dudau C, Connor S. (2019). Growth and late detection of post-operative cholesteatoma on long term follow-up with diffusion weighted magnetic resonance imaging (DWI MRI): A retrospective analysis from a single UK centre. Otol Neurotol. 40: 638-644.

## 37.1 OX40 and OX40L: Can signalling between these genetic risk factors identify cellular targets for therapy in SLE?

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**Project Description:**

The signal between OX40 (*TNFRSF4*) and its unique ligand OX40L (*TNFSF4*) is a crucial checkpointmodulator within the immune system. Both molecules are genetic risk-factors for multiple autoimmune diseases, including Systemic Lupus Erythematosus (SLE) and risk-alleles at both loci correlate with elevated gene expression *in-cis*. OX40 is primarily expressed on activated $CD4^{+}$-T celltypes whereas OX40L is predominantly expressed activated antigen presenting cell-types (APCs). The signal generated between OX40 and OX40L can either boost the immune response from Th1, Th2, Th9 and Tfh cells, but inhibit that from Treg, Th17, Tfr and Tr1 cells. Detailed protein/transcriptomic phenotyping of co-cultures between OX40+ and OX40L+ cell-types will reveal the cell-pairings creating the strongest OX40-OX40L regulatory signal. By establishing which cellpairs respond most strongly to an OX40-OX40L signal block (OX40:Fc fusion protein) and testing it in mouse models of lupus, we may determine a pivotal immune checkpoint which could lead to a celltype specific therapy for autoimmune disease.

Techniques and skills: transcript profiling in immune cells (scRNA-Seq); eQTL analysis; serum protein levels (ELISAs); immune profiling and cell-sorting (flow cytometry); immune cell co-culture

Main Objectives for PhD:
*Year 1:* Characterise the genetic contribution to the OX40+ and OX40L+ immune cell populations in SLE cases and healthy controls
*Year 2-3:* Determine which pair of immune cells shows the strongest OX40-OX40L signal and the greatest perturbation in cytokine production, cell proliferation and/or transcriptional profile *Year 3:* Establish the immune cell-pairs showing the greatest response to blocking the OX40-OX40L signal in human and mouse cells

**<u>One</u> representative publication from <u>each co-supervisor</u>:**

Cortini, A., Ellinghaus, U., Malik, T. H., Cunninghame Graham, D. S., Botto, M. & Vyse, T. J., B cell OX40L supports T follicular helper cell development and contributes to SLE pathogenesis (2017). *Annals Rheum. Dis.* 76: 2095-2103

Cunninghame Graham, D. S., Graham, R. R., Manku, H., Wong, A. K., Whittaker, J. C., Gaffney, P. M.,

Moser, K. L., Rioux, J. D., Altshuler, D., Behrens, T. W. & Vyse, T. J. Polymorphism at the TNF superfamily gene *TNFSF4* confers susceptibility to systemic lupus erythematosus (2008). *Nat. Genet.* 40: 83-89

## 38.1 Characterising transcriptional control of Brachyury in breast cancer.

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**Project Description:**

The transcription factor, Brachyury, is expressed during embryonic development, but not in the adult body. However, *Brachyury* expression is re-activated in several cancers, where it plays a role in metastasis through regulating epithelial-to-mesenchymal transition. High levels of *Brachyury* in breast cancer are correlated with late stages of the disease and distant metastasis, and studies in mouse show that knocking down *Brachyury* expression limits tumour size and metastasis.

However, nothing is known about how *Brachyury* expression is activated and maintained in breast cancer cells. Using 4C-seq, we have recently identified genomic regions that may regulate *Brachyury* expression, and this project aims to functionally validate the requirement for these putative control regions. We hypothesise that characterising the regions that control *Brachyury* expression will provide novel options for the treatment of breast cancer.

The student will analyse publicly available histone ChIP-seq data to identify 4C-seq regions that overlap active or inactive genomic regions (Year 1). These data will be used in a CRISPR/Cas9 system, to screen these regions for their requirement to drive *Brachyury* expression in breast cancer cells (Year 1 and 2). A screen for sufficiency, using reporter assays, will also be performed (Year 2). Subsequent bioinformatic, mutational and pathway analysis of the identified regions will further characterise the regulation of *Brachyury* (Year 3). The student will be trained in bioinformatics, molecular biology, cell culture, CRISPR guide RNA design, cell transfection and data analysis.

**One representative publication from each co-supervisor:**

Nelson A. C., Cutty S.J., Gasiunas S.N., Deplae I., Stemple D.L., Wardle F.C. (2017). In vivo regulation of the zebrafish endoderm progenitor niche by T-box transcription factors. Cell Reports, 19:2782-95.

Mario De Piano, Valeria Manuelli, Giorgia Zadra, Jonathan Otte, Per-Henrik D. Edqvist, Fredrik Pontén, Salpie Nowinski, Athanasios Niaouris, Anita Grigoriadis, Massimo Loda, Mieke Van Hemelrijck and Claire M. Wells (2020). Lipogenic signaling modulates prostate cancer cell adhesion and migration via modification of Rho GTPases. Oncogene 39:3666–3679 doi.org/10.1038/s41388020-1243-2

## 39.1 Pathogenic mast cells in chronic rhinosinusitis with nasal polyps.

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**Project Description:**

Mast cells (MC) play an important role in pathogenesis of Type-2 mediated respiratory diseases such as asthma and chronic rhinosinusitis with nasal polyps (CRSwNP). We studied immune cells obtained from nasal polyps and found increased percentages of MC expressing IL-17RB (receptor for IL-25), identifying a potentially novel MC subpopulation. Our aim is to determine functional mechanisms of IL-17RB+ MC contribution to the pathogenesis of CRSwNP. The project will allow us for the first time to quantitatively and qualitatively determine subpopulations of human airway MCs and determine their role in inflammatory Type-2 responses.

Year 1: The student will use experimental techniques for analysis of human MC lines and primary MCs isolated from polyp tissues, including tissue digestion, cell culture and flow cytometry. Subpopulations will be quantified, phenotyped and cell sorted for further analysis.

Year 2: As data on human MC heterogeneity are very limited the student will use an unbiased approach to evaluate airway MC heterogeneity at the single cell transcriptomic and chromatin levels in nasal polyps, followed by functional analysis of identified subpopulations.

Year 3: MCs are activated in CRSwNP by the local environment, including IL-25 and IL-33. To test this hypothesis, the student will first study cell responses to cytokines in human MC cell line, LAD2, overexpressing IL-17RB, followed by functional analysis of selected subpopulations of primary MCs using a range of molecular and cellular biology techniques such as RT-PCR, lentiviral delivery system, Western blotting, flow cytometry, intracellular calcium signalling, cell degranulation and chemotaxis.

**One representative publication from each co-supervisor:**

Xia J, Abdu S, Maguire TJA, Hopkins C, Till SJ, Woszczek G. Prostaglandin D2 receptors in human mast cells. Allergy. 2020 Jun;75(6):1477-1480.

Lam EP, Kariyawasam HH, Rana BM, Durham SR, McKenzie AN, Powell N, Orban N, Lennartz-Walker M, Hopkins C, Ying S, Rimmer J, Lund VJ, Cousins DJ, Till SJ. IL-25/IL-33-responsive TH2 cells characterize nasal polyps with a default TH17 signature in nasal mucosa. J Allergy Clin Immunol. 2016 May;137(5):1514-24

## 40.1 Development of a breakthrough anti-fibrotic gene therapy to improve surgical outcomes and reduce re-admission rates for patients with severe glaucoma.

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**Project Description:**

Glaucoma is the leading cause of irreversible blindness and affects 76 million people worldwide. Glaucoma filtration surgery is the mainstay of surgical treatment in glaucoma, but the failure rate is high at 50% after 5 years. This project focuses on the pre-clinical development of a novel targeted gene therapy to increase the surgical success in glaucoma by reducing post-surgical scarring, without exposure to cytotoxic drugs like mitomycin-C.

Our lab has developed innovative lipid and peptide-based nanoparticle encapsulation methods, which protect RNA molecules from degradation and deliver them directly to the affected site for targeted silencing of pathogenic genes.

We aim to rapidly progress the development of the gene therapy and conduct Good-LaboratoryPractice (GLP) toxicology/efficacy studies to provide vital data for future human trials.

Project objectives:
1) In Year 1, the student will optimise a tailored mix of peptides/lipids encapsulating a genetic sequence enabling active targeted uptake in human conjunctival fibroblasts. Skills learned: Drug formulation, cell culture, real-time qPCR.

2) In Years 2-3, the student will develop a toxicological report, detailing the *in vivo* efficacy and toxicological profile of the optimised RNA therapeutics in the experimental rabbit model of glaucoma surgery. Skills learned: Examining animals, histology, fluid sampling for high-performance liquid chromatography.

3) In Years 3-4, the student will develop manufacturing/formulation routes for the gene therapy, providing a repeatable and safe manufacturing process. Skills learned: Gain insight into the translational pipeline, including collaboration with the GMP lab at King's College London, MHRA approval process, and defining an effective route to market.

**<u>One</u> representative publication from <u>each co-supervisor</u>:**

Fernando O, Tagalakis AD, Awwad S, Brocchini S, Khaw PT, Hart SL, Yu-Wai-Man C. Development of targeted siRNA nanocomplexes to prevent fibrosis in experimental glaucoma filtration surgery. *Mol Ther*. 2018 Dec; 26(12): 2812-2822.

Craig JE, Han X, Qassim A, NEIGHBOURHOOD consortium, UK Biobank Eye and Vision Consortium, Hammond CJ, Mackey DA, Mitchell P, Lotery AJ, Wiggs JL, Hewitt AW, MacGregor S. Multitrait analysis of glaucoma identifies new risk loci and enables polygenic prediction of disease susceptibility and progression. *Nat Genet*. 2020 Feb; 52(2): 160-166.

## 41.1 Improving muscle function in a skeletal muscle wasting disorder.

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**Project Description:**

Muscular dystrophies are characterised by muscle weakness and wasting. One such disorder, Facioscapulohumeral muscular dystrophy (FSHD), is caused by ectopic expression of a transcription factor called DUX4. FSHD muscle cells are sensitive to damage caused by free radicals (oxidative stress), and treatment of FSHD patients with anti-oxidants vitamin E, vitamin C, zinc, and selenomethionine improves some muscle function measurements (clinicaltrials.gov: NCT01596803) (doi:10.1016/j.freeradbiomed.2014.09.014). Analysis of our extensive FSHD gene expression data from muscle and immune cells has implicated several mediators of oxidative stress and mitochondrial function, along with pathways controlling inflammation. For example, we found that the PGC1alpha/ERRalpha axis is suppressed in FSHD and showed that compounds/ nutritional supplements targeting this pathway improved muscle formation (Banerji et al., 2019: doi:10.1093/hmg/ddy405).

Hypothesis: Improving protection against oxidative stress and inflammation will improve muscle function in FSHD.

Objectives:
Year 1: Use bioinformatics to examine gene expression data from FSHD. Examine expression dynamics and manipulate pathways identified as central to oxidative stress and inflammation in FSHD via knockout/knockdown (CRISPR/siRNA/antagonists) and overexpression (viral-mediated delivery, agonists) strategies in FSHD patient cells.
Year 2: Determine relationship of selected pathways to DUX4 using both in vitro and in vivo models.
Year 3/4: Screen drugs/nutritional supplements that can affect these pathways to determine if protection against oxidative stress and inflammation in FSHD improves muscle function and repair.

Skills training: Molecular Biology (e.g. cloning, CRISPR), Cell Biology (mouse/human cell culture, retroviral-transduction, siRNA-mediated gene-knockdown), Animal Models, Gene Expression/Protein Analysis (RT-qPCR, Western blotting, immunolabeling), Imaging/Time-Lapse using state-of-the-art confocal/multiphoton microscopy and Bioinformatics.

**One representative publication from each co-supervisor:**

Banerji C.R.S, Panamarova M., Pruller J., Figeac N., Hebaishi H., Fidanis E., Saxena A., Contet J., Sacconi S., Severini S. and Zammit P.S (2019). Dynamic transcriptomic analysis reveals suppression of PGC1α/ERRα drives perturbed myogenesis in facioscapulohumeral muscular dystrophy. *Human Molecular Genetics* 28, 1244-1259 (doi: 10.1093/hmg/ddy405)

Lange, S., K. Gehmlich, A.S. Lun, J. Blondelle, C. Hooper, N.D. Dalton, E.A. Alvarez, X. Zhang, M.-L. Bang, Y.A. Abassi, C.G. dos Remedios, K.L. Peterson, J. Chen and E. Ehler (2016): MLP and CARP are linked to chronic PKCalpha signaling in dilated cardiomyopathy. Nat. Commun. 7:12120. doi: 10.1038/ncomms12120.

## 42.1 Host-microbial interactions in periodontal health and disease: Exploring the role of bacterial and immunological biomarkers in personalised dental care.

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**Project Description:**

Current diagnostic criteria for periodontitis are based on detection of historic tissue destruction and leave no opportunity to predict future activity of the disease or to formulate an appropriate treatment plan specific to each individual patient. Our aim is to develop a rapid and reliable test based on oral bacterial lipid-A profile, which can be used routinely by the dental care professionals and the wider public in order to maintain and improve oral health and quality of life and to encourage access to dental care and reduce health disparities. The test would be made in two versions: 1) for home, self-testing for early detection and prevention of periodontal disease before significant tissue destruction occurs, and 2) for the chair-side use by dental professionals for personalised, point-of-care periodontal therapy.

Hypothesis: Subgingival and salivary endotoxin activity (determined by the lipid A chemical composition and the amount of LPS) can be used as a reliable, bacterially derived biomarker and a risk assessment tool for prevention of periodontal diseases and personalised periodontal care.

Objectives:

1. To determine subgingival and salivary lipid-A profiles in persons with healthy periodontium, gingivitis and periodontitis patients using Mass-spectrometry.
2. To examine endotoxin activity and inflammatory potential of subgingival and salivary lipid-A extracts
3. To compare subgingival and salivary lipid-A profiles, lipid-A bioactivity and its endotoxin potential in gingivitis and periodontitis patients before and after non-surgical periodontal therapy.
4. To devise and optimise a novel, rapid, convenient and reliable subgingival and salivary test for periodontal risk-assessment.

**One representative publication from each co-supervisor:**

Mcilwaine C, Strachan A, Harrington Z, Jerreat M, Belfield LA, Sandor V, Foey A, Zaric S. Comparative analysis of total salivary lipopolysaccharide chemical and biological properties with periodontal status. Arch Oral Biol. 2020 Feb;110:104633.

Houghton, JW, Carpenter, GH, Joachim, H., Pesaro, M., Lynham, S., Proctor, GB. Agonists of orally expressed TRP channels stimulate salivary secretion and modify the salivary proteome. Mol. Cell. Proteomics. DOI:10.1074/mcp.RA120.002174 (2020).