Theme 1
Molecules, Cells and the Basis for Disease

2020/2021

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

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

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1.1 Using single cell transcriptomics to uncover the mechanisms of beta cell expansion during pregnancy.

Co Supervisor 1A: Dr Marika Charalambous

Research School/ Division or CAG: Medical and Molecular Genetics
Email: marika.charalambous@kcl.ac.uk
Website: https://www.kcl.ac.uk/people/marika-charalambous

Co Supervisor 1B: Dr Rocio Sancho
Research School/ Division or CAG: Centre for Stem Cells and Regenerative Medicine
Email: rocio.sancho@kcl.ac.uk
Website: Rocio Sancho – Website KCL

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 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.

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

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.

2.1 Nanoneedle-mediated engineering of extracellular vesicles for targeted gene delivery.

Co Supervisor 1A: Ciro Chiappin
Research School/ Division or CAG: Centre for Craniofacial and Regenerative Biology

Email: ciro.chiappini@kcl.ac.uk
Website: chiappinilab.com

Co Supervisor 1B: Francesco Dazzi
Research School/ Division or CAG: Cancer and Pharmacological Sciences/Comprehensive Cancer Centre
Email: francesco.dazzi@kcl.ac.uk
Website: dazzilab.com

Project Description:

This project combines bionanomaterials, cell biology, drug formulation and *in vivo* models to engineer extracellular vesicles. EVs have emerged as efficient nanocarriers for drug delivery, thanks to their exceptional ability to evade the immune system and to actively target disease sites. Yet their potential is hampered by the limited and inefficient strategies available to load desired biologicals within EVs. With our nanoneedle platform, we have developed an efficient mechanism to deliver payloads to cells by modulating multiple endocytic pathways simultaneously. Here we leverage this endocytic modulation to load the L807 GSK3 inhibitor peptide within EVs. L807 is a highly specific GSK3 inhibitor, capable of activating the Wnt/βcat pathway with significantly lower side-effects than small molecules.

We will culture mesenchymal stem cells (MSCs) over nanoneedles loaded with mesoporous silica nanoparticles containing L807. The nanoneedles will induce the MSCs to uptake. The nanoparticles will protect the L807 from peptidases through the endocytosis and subsequent exocytosis. We will isolate and characterise the properties of EVs *in vitro* and evaluate Wnt activation using Axin2 reporter models *in vitro* and *in vivo*. This approach provides a strategy for the targeted, specific in vivo modulation of the Wnt pathway, of primary importance in development and regeneration. Our preliminary data shows the feasibility of our approach in introducing nanoparticles with payload within EV with high efficiency.

Y1: Engineer EV payload composition. Identify the conditions for mesoporous silica loading and subsequent trafficking for exocytosis.

Y2-3: Isolate, purify and characterise EVs. Evaluate efficacy *in vitro*.

Y3-4: Evaluate biodistribution, stability and efficacy *in vivo*.

One representative publication from each co-supervisor:

1. S. Gopal, C. Chiappini*, J. Penders, V. Leonardo, H. Seong, S. Rothery, Y. Korchev, A. Shevchuk*, M. Stevens*, Porous Silicon Nanoneedles Modulate Endocytosis to Deliver Biological Payloads, Adv. Mater. 31 1806788 (2019). *Corresponding Author.

2. Galleu A Riffo-Vasquez Y, Trento C, Lomas C, Dolcetti L, Cheung TS, von Bonin M, Barbieri L, Halai K, Ward S, Weng L, Chakraverty R, Lombardi G, Watt FM, Orchard K, Marks DI, Apperley J, Bornhauser M, Walczak H, Bennett C, and Dazzi F. Perforin-dependent apoptosis in mesenchymal stromal cells is required to initiate host-mediated *in vivo* immunomodulation. Science Translational Medicine 2017 *vol 9, Issue 416, eaam7828*

3.1 Function of cell stress-associated RNA-binding proteins in cancer.

Co Supervisor 1A: Maria R (Sasi) Conte
Research School/ Division or CAG: Randall Centre for Cell and Molecular Biophysics, School of Basic and Medical Biosciences
Email: sasi.conte@kcl.ac.uk
Website: https://www.kcl.ac.uk/people/sasi-conte

Co Supervisor 1B: Peter Parker
Research School/ Division or CAG: School of Cancer and Pharmaceutical Sciences; Cancer CAG.
Email: peter.parker@kcl.ac.uk
Website: https://www.kcl.ac.uk/people/parker

Project Description:

Dysregulation of protein-protein and protein-RNA networks are prominent features of cancer. We have recently discovered that two RNA-binding protein (RBPs), namely LARP4A and LARP4B, participate in the spatio-temporal regulation of protein synthesis, affecting cancer cell motility and division. We also have exciting new data that LARP4B, alongside another RBP called SERPB1, are substrates of a central kinase regulating the cell cycle under stress conditions, protein kinase Cε (PKCε). LARP4A/B and SERBP1 contain intrinsically disordered regions that are involved in the formation of amyloid-like granules in the cell. These are hubs of RBPs and RNA, often regulated by signalling events (*e.g.* phosphorylation) and causally associated to cancer. Of relevance, we have identified PKCε regulated, protein/RNA-containing structures (M-bodies) in mitosis that are associated with protection from non-disjunction and division failure.

The aim of this project is to elucidate the molecular mechanisms underlying the formation of these pathological amyloid-type ultrastructures, seeking to understand their impact on cancer cell motility, growth/division. Objectives include: (i) characterise the detailed molecular associations between LARP4A/B, RNA and their regulatory protein network (involving polyA-binding protein PABP, RACK1, PKCε and SERBP1) (year 1-2/3); (ii) assess the role of conformational disorder for the formation of RBP networks (year 2-3); (iii) determine the function of these networks in cell migration and the cell cycle (year 1-3); (iv) reveal the translational circuitries regulated by LARP4A/B that underlie their specific impact on cell behavior (year 2-3/4).

This interdisciplinary work will combine *in vitro* studies of RBP structure and modes of interaction with RNAs and partner proteins with cell analyses, taking advantage of the complementary expertise of the supervisors. Training and experience will be provided in the use of molecular biology, protein/RNA biochemistry/biophysics (*e.g.* EMSA, ITC, CD, H-DX MS), structural and chemical biology (NMR, SAXS etc.), bioinformatics (analysis of RNA-seq, proteomics data), codon expansion technology, confocal and video microscopy, cell biology (*e.g.* CRISPR/Cas9 etc.). The strong link between the supervisors and the Crick institute also offers opportunities to acquire additional expertise and training.

This work has the potential to identify new cancer diagnostic tools and treatment targets and the assessment and development in these directions would be a part of the PhD experience.

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

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., Epub, doi: 10.1093/nar/gkz144*

Pike, T., Brownlow, N., Kjaer, S., Carlton, J., Parker, P.J. (2016) PKCε switches Aurora B specificity to exit the abscission checkpoint *Nat. Comm. 7:13853.*

4.1 Development of glycan-binding broadly neutralizing antibody responses in HIV-1 infection.

Co Supervisor 1A: Dr Katie J Doores
Research School/ Division or CAG: SIMS
Email: katie.doores@kcl.ac.uk
Website:https://www.kcl.ac.uk/lsm/research/divisions/diiid/departments/infectious/research/doores/index

Co Supervisor 1B: Dr Julie Fox
Research School/ Division or CAG: SIMS
Email: julie.fox@kcl.ac.uk
Website:
https://www.kcl.ac.uk/lsm/research/divisions/diiid/departments/infectious/research/juliefox.aspx

Project Description:

An HIV vaccine is desperately needed to prevent new HIV infections worldwide. Approximately 10-30% of HIV infected individuals generate antibodies that are capable of neutralizing a broad range of HIV isolates and these neutralizing antibodies (nAbs) have been shown to protect against SHIV challenge in Macaque models. Isolation and characterisation of nAbs has revealed regions of the HIV envelope glycoprotein, gp120/gp41, that are targeted by the host immune response during infection and re-eliciting these nAbs may be a key step for a successful HIV vaccine. Gp120 is heavily glycosylated with host-derived N-linked glycans and although glycans were previously thought to shield conserved protein regions from the immune system, we have shown that many of the most broad and potent HIV nAbs bind directly to glycans highlighting them as important targets for HIV vaccine design.

Immunization with recombinant gp120/gp41 does not generate glycan-binding nAbs. Therefore, using unique longitudinal clinical samples from individuals acutely infected with HIV in the SPARTAC study (N Engl J Med 2013;368:207-17), we will investigate the development of glycan-binding HIV broadly neutralizing antibodies (bnAbs) during infection using *in vitro* neutralization assays, antigen-specific B cell sorting and antibody cloning, glycan array analysis, next generation sequencing of antibody genes and HIV Envelope. We will determine how the viral Envelope evolution guides and directs bnAb development in these HIV-infected individuals and the role of pre-existing anti-glycan antibody responses. Ultimately these studies will be used to design immunogens and immunization strategies aimed at re-eliciting these bnAbs through vaccination.

One representative publication from each co-supervisor:

1. L. M. Walker,* M. Huber,* **K. J. Doores**,* E. Falkowska, R. Pejchal, J.-P. Julien, S.-K. Wang, A. Ramos, P. Y. Chan-Hui, M. Moyle, J. L. Mitcham, P. W. Hammond, O. A. Olsen, P. Phung, S. Fling, C.-H. Wong, S. Phogat, T. Wrin, M. D. Simek, Protocol G Principal Investigators, W. C. Koff, I. A. Wilson, D. R. Burton, P. Poignard, Broad neutralization coverage of HIV by multiple highly potent antibodies, ***Nature***, 2011, 477, 466-470.

2. J. Tiraboschi , S. Ray, K. Patel, A. Teague, M. Pace, P. Phalora, N. Robinson, E. Hopkins, J. Meyerowitz, Y. Wang, J. Cason, S. Kaye, J. Sanderson, P. Klenerman, S. Fidler, J. Frater, **J. Fox**, The impact of immunoglobulin in acute HIV infection on the HIV reservoir: a randomized controlled trial, ***HIV Med***, 2017, doi: 10.1111/hiv.12524.

5.1 The impact of ageing on epigenetic regulators of cardiomyocyte and vascular cell differentiation.

Co Supervisor 1A: Georgina Ellison-Hughes
Research School/ Division or CAG: School of Basic & Medical Biosciences/Centre for Human and Physiological Sciences
Email: georgina.ellison@kcl.ac.uk
Website: https://www.kcl.ac.uk/people/georgina-ellison-hughes

Co Supervisor 1B: TBC
Research School/ Division or CAG:
Email:
Website:

Project Description:

Ageing is the greatest risk factor for many life-threatening disorders, including cardiovascular disease, neurodegeneration and cancer. The structure and function of the heart and cardiovascular system deteriorates considerably with age, and the ability of the heart to repair and regenerate is greatly compromised with increased age. As the majority of cardiovascular disease patients in need of regenerative therapies are of advanced age, it's important we understand how ageing impacts the regeneration of new cardiomyocytes and vascular cells.

In this project you will discover novel genes and downstream events in cardiomyocytes and vascular cells from different aged subjects. You will determine whether there are any chromatin changes as a result of ageing.

Year 1: You will derive cardiomyocytes and vascular cells from cardiac stem/progenitor cells *in vitro* from different aged donors (young, middle-aged, old). Then you will use RNA seq to discover novel up- and down-regulated genes at different donor age.

Year 2: You will use laser micro-dissection microscopy to dissect out cardiomyocytes and vascular cells from tissue sections of different donor age, and isolate total RNA for RNA seq. These data from the dissected cells will be compared to the in vitro differentiated cells.

Year 3/4: You will use –omics technologies to determine chromatin structure of cardiomyoctes and vascular cells at different ages.

Skills and rotation project training: You will receive training in some of the advanced technologies available – RNA seq and -omics technologies, laser micro-dissection microscopy, derivation of cardiomyocytes and vascular cells from stem cells *in vitro*.

One representative publication from each co-supervisor:

Vicinanza C, Aquila I, Scalise M, Cristiano F, Marino F, Cianflone E, Mancuso T, Marotta P, Sacco W, Lewis FC, Couch L, Shone V, Gritti G, Torella A, Smith AJ, Terracciano CMN, Britti D, Veltri P, Indolfi C, Nadal-Ginard B, **Ellison-Hughes GM**, Torella D. (2017) Adult Cardiac Stem Cells are Multipotent and

Robustly Myogenic: c-kit Expression is Necessary but not Sufficient for their Identification. *Cell Death & Differentiation, Aug 11. doi: 10.1038/cdd.2017.130.*

6.1 Novel mechanisms for the regulation of contractility in the heart.

Co Supervisor 1A: Luca Fusi
Research School/ Division or CAG: BMBS/ Randall Centre
Email: luca.fusi@kcl.ac.uk
Website: https://www.kcl.ac.uk/research/profile/fusi-group

Co Supervisor 1B: Malcolm Irving
Research School/ Division or CAG: BMBS/Randall Centre
Email: malcolm.irving@kcl.ac.uk
Website:
https://www.kcl.ac.uk/lsm/research/divisions/randall/research/sections/signalling/irving/index.aspx

Project Description:

Precise dynamic regulation of contraction and relaxation of cardiac muscle is essential for the normal function of the heart. Defects in this dynamic regulation lead to reduced cardiac output and, ultimately, to heart failure. Our limited understanding of the basic molecular mechanisms responsible for heart failure currently limits the development of new therapies for heart diseases. We have recently discovered that the strength of contraction in the heart is controlled by regulatory mechanisms that modulate the activation of the myosin motors, in addition to the calcium-dependent activation of the actin filaments.

The aim of this project is to investigate the dynamic regulation of the myosin motors in cardiac muscle cells during the heartbeat.

Year 1: The student will receive a training in molecular biology for the expression, purification and labelling of muscle proteins for fluorescence polarisation experiments. He/She will learn to isolate biological samples from animal models, in which the fluorescent proteins will be introduced, for contractile and fluorescence measurements on cardiac muscle cells.

Year 2: Having gained competence in all the necessary techniques and with an understanding of the relevant background, the student will focus on the development of a cardiac muscle preparation in which the physiological calcium transient can be mimicked in combination with exchange of labelled protein components in order to use time-resolved fluorescence polarisation to measure the changes in myosin conformation associated with force development and relaxation.

Year 3: The student will use the skills and methods developed in the previous years and the acquired ability to meet the deadlines to successfully conclude the investigation of the molecular mechanisms that control the contraction of cardiac muscle cells and their disruption in heart diseases.

One representative publication from each co-supervisor:

Fusi, L., Brunello, E., Yan, Z., Irving, M. Thick filament mechano-sensing is a calcium-independent regulatory mechanism in skeletal muscle. *Nat Commun* **7**, 13281 (2016).

Kampourakis, T., Y. B. Sun, and M. Irving. (2016). Myosin light chain phosphorylation enhances contraction of heart muscle via structural changes in both thick and thin filaments. *Proc Natl Acad Sci (USA)* 113:E3039-E3047.

7.1 The effect of lipid composition on the mechanostransduction of individual live cells.

Co Supervisor 1A: Prof Sergi Garcia-Manyes
Research School/ Division or CAG: Physics/Randall Division
Email: sergi.garcia-manyes@kcl.ac.uk
Website: http://garcia-manyeslab.org/

Co Supervisor 1B: Prof Ulrike Eggert
Research School/ Division or CAG: Randall Division/Chemistry
Email: ulrike.eggert@kcl.ac.uk
Website:
http://www.kcl.ac.uk/biohealth/research/divisions/randall/research/sections/motility/eggert/index.aspx

Project Description:

Are the lipids forming the plasma membrane, organelles and nuclear envelope (NE) dynamically modified under mechanical stress? Lipids and proteins are key components of membranes, yet most of the effort to understand mechanotransduction has focused on proteins alone. First, we will explore whether the lipidome changes in the plasma membranes, organelles and NE of cells exposed to mechanical stress. We will subject cultured cells to substrates of different stiffness, and extract their nuclei. Plasma membrane and nuclear lipids will be extracted and analysed by MS to determine their lipidomic profiles. In parallel, we will use Atomic Force Microscopy (AFM) in combination with magnetic tweezers cell stretching experiments to probe the mechanical properties of plasma and nuclear membranes.

We will investigate the effect of mechanical forces on the lipid composition of cells and isolated nuclei and organells. The student will gain expertise in single cell AFM and magnetic tweezers characterisation, 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).

One representative publication from each co-supervisor:

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.

8.1 Molecular understanding of outer membrane vesicle formation in *Porphyromonas gingivalis.*

Co Supervisor 1A: Dr James Garnett
Research School/ Division or CAG: Centre for Host-Microbiome Interactions, FoDOCS
Email: james.garnett@kcl.ac.uk
Website: https://www.kcl.ac.uk/research/profile/garnett-lab-fodocs

Co Supervisor 1B: Prof Mike Curtis
Research School/ Division or CAG: Centre for Host-Microbiome Interactions, FoDOCS
Email: mike.curtis@kcl.ac.uk
Website: https://www.kcl.ac.uk/people/mike-curtis

Project Description:

Bacteria produce outer-membrane vesicles (OMVs) via blebbing of the outer-membrane, although how they form is unclear. OMVs are decorated with proteins and polysaccharides and are important for progression of bacterial disease. *Porphyromonas gingivalis* is a Gram-negative pathogenic bacterium of the oral cavity and causes chronic periodontitis. It uses a type-IX secretion system (T9SS) to export virulence factors, which are then covalently attached to the cell surface via a specific type of liposaccharide (A-LPS) and also sorted into OMVs. Anchorage of A-LPS to the outer-membrane is mediated through the lipid A component of the molecule. We have determined that the T9SS regulates the phosphorylation status of lipid A via a lipid A phosphatase (LpxE), and this is essential for correct OMV formation. Gaining molecular insight here will allow us to understand new biological processes but may also present novel drug targets for developing new antibacterial compounds. The specific aims of this PhD project are to:

Aim-1: *Decipher how A-LPS is transported to LpxE (Year 1)*

Aim-2: *Determine structure(s) of A-LPS transporter(s) (Years 2-3)*

Aim-3: *Determine structures of T9SS/LpxE/A-LPS complexes (Years 1-3)*

Aim-4: Verify models with mutations in *P. gingivalis that disrupt OMV production (Year 3)*

This project involves anaerobic culturing of *P. gingivalis*, creating mutant strains and OMV isolation. Proteins will be expressed in *Escherichia coli* and purified for structural biology studies. The Garnett/Curtis laboratories have vast experience working with these techniques and studying *P. gingivalis* and bacterial secretion. Additional training will be provided through internal/external workshops and national/international scientific meetings.

One representative publication from each co-supervisor:

White, R.C., Gunderson, F.F., Tyson, J.Y., Richardson, K.H., Portlock, T.J., Garnett, J.A. & Cianciotto, N.P. (2018). Type II secretion-dependent aminopeptidase LapA and acyltransferase PlaC are

redundant for nutrient acquisition during Legionella pneumophila intracellular infection of amoebas. **mBio** 9, e00528-18

Rangarajan M, Aduse-Opoku J, Hashim A, McPhail G, Luklinska Z, Haurat MF, Feldman MF, Curtis MA. (2017) LptO (PG0027) Is Required for Lipid A 1-Phosphatase Activity in Porphyromonas gingivalis W50. **J Bacteriol**. 199(11): e00751-16. doi: 10.1128/JB.00751-16

10.1 A Novel Approach to limit the tumorigenic potential of Osteosarcoma.

Co Supervisor 1A: Shukry James Habib
Research School/ Division or CAG: Centre for Stem Cells and Regenerative Medicine
Email: shukry.habib@kcl.ac.uk
Website: www.habiblab.org

Co Supervisor 1B: Prof Jeremy Green
Research School/ Division or CAG: Craniofacial Development and Stem Cell Biology
Email: jeremy.green@kcl.ac.uk
Website: https://kclpure.kcl.ac.uk/portal/jeremy.green.html

Project Description:

The bone cancer Osteosarcoma is the second leading cause of cancer-related deaths in paediatric patients. Despite surgery and chemotherapy, long-term survival rates for patients diagnosed with osteosarcoma have not improved over the last 30 years. Osteosarcoma stem cells (OSCS) derive the growth of tumour. The levels of Lrp5, the receptor of Wnt ligands, are statistically correlated with poor prognosis and Wnt signalling is a therapeutic target for blocking tumorogensis.
This project will investigate the cell division of OSCS, and engineer strategies to polarise them in order to direct them to divide asymmetrically, limit their proliferation potential and control cellular fate. We hypothesise that the Wnt signalling pathway could be employed for controlling the cellular polarity and division.

The Habib lab has engineered localised Wnt niches that can induce asymmetric cell division of embryonic and bone stem cells. The combination of our bioengineering approaches with cell polarity expertise (Green lab) provide grounds to explore the mechanistic regulation of OSSC division and opportunities to limit their tumorigenic potential.

1st -2nd year: Culturing and characterising OSCS. Employing 3D microscopy to study the division OSCS by establishing a molecular segregation map for cell polarity proteins, Wnt pathway components and cell fate markers. Purification of Wnt proteins will also be done.

3nd- 4th year: Engineering localised Wnt niches in 3D biomaterials and testing their effect on OSCS proliferation and invasion. Flow cytometery to separate between the daughter cells of OSCSs and investigating their tumorigenic potential in in vitro and in vivo transplantation assays.

One representative publication from each co-supervisor:

1) Habib SJ et al A localized Wnt signal orients asymmetric stem cell division in vitro. *Science* 2013

2) Tabler JM, Yamanaka H, Green JB. PAR-1 promotes primary neurogenesis and asymmetric cell divisions via control of spindle orientation. *Development* 2010

11.1 Regulation of the integrated stress response by RNA modifications.

Co Supervisor 1A: Dr Michelle Holland
Research School/ Division or CAG: School of Basic and Medical Biosciences
Email: michelle.holland@kcl.ac.uk
Website: https://www.kcl.ac.uk/people/michelle-holland https://www.hollandlaboratory.org/

Co Supervisor 1B: Chad Swanson
Research School/ Division or CAG: School of Immunology & Microbial Sciences
Email: chad.swanson @kcl.ac.uk
Website: https://www.kcl.ac.uk/research/profile/swanson-lab

Project Description:

The integrated stress response is a pathway that responds to diverse environmental stimuli such as virus infection, nutrient starvation, ER stress, heat stress and others. Upon environmental stress, one or more of a family of kinases are activated which leads to the repression of protein synthesis, stress granule induction and activation of specific genes. This inhibits viral infection and helps restores cellular homeostasis once the stress has ended. One of the major knowledge gaps in this pathway is how RNA modifications regulate this response. RNA can be modified by methylation, acetylation or other chemical modifications and this regulates the function of the RNA. Both mRNAs and non-coding RNAs are modified, but only recently have the technologies been developed to analyse this in a genome-wide manner. Elucidating how the integrated stress response is regulated may provide the foundation for novel therapeutic interventions against many diseases, including viral infection and cancer.

In this project, RNA-seq technologies will be used to analyse how mRNAs and non-coding RNAs are regulated by the integrated stress response in wild type cells and cells that have the enzymes that add specific RNA modifications such as adenosine methylation (m6A), cytidine methylation (m5C), cytidine acetylation (ac4C) and 2-O-ribose methylation knocked out by CRISPR-Cas9-mediated genome editing. This will be correlated with the effect on viral replication and cell viability in response to nutrient starvation, heat shock and other stresses. Overall, this project will determine how specific post-transcriptional RNA modifications regulate the integrated stress response to diverse environmental stimuli.

One representative publication from each co-supervisor:

Holland ML, Lowe R, Caton PW, Gemma C, Carbajosa G, Danson AF, Carpenter AAM, Loche E, Ozanne SE, Rakyan VK (2016) *Science* 10.1126/science.aaf7040 Early life nutrition modulates the epigenetic state of specific rDNA genetic variants in mice.

KHNYN is essential for the zinc finger antiviral protein (ZAP) to restrict HIV-1 containing clustered CpG dinucleotides. Ficarelli M, Wilson H, Pedro Galão R, Mazzon M, Antzin-Anduetza I, Marsh M, Neil SJ, **Swanson CM**. Elife. 2019 Jul 9;8.

12.1 Transcriptional control of stem cell decisions during muscle growth: quiescence, proliferation, fusion or new fibre formation?

Co Supervisor 1A: Prof Simon M Hughes
Research School/ Division or CAG: Randall Centre for Cell and Molecular Biophysics
Email: simon.hughes@kcl.ac.uk
Website:
https://www.kcl.ac.uk/lsm/research/divisions/randall/research/sections/signalling/hughes/members

Co Supervisor 1B: Dr Yaniv Hinits
Research School/ Division or CAG: Randall Centre
Email: yaniv.hinits@kcl.ac.uk
Website: https://kclpure.kcl.ac.uk/portal/yaniv.hinits.html

Project Description:

Muscles grow in response to high force exercise, but how the muscle senses force and how this then triggers growth is unknown. We recently developed a zebrafish larva model in which a single bout of force generation triggers 30% muscle growth in 24 hours. We know muscle stem cells (MSCs) respond to the growth stimulus by making new fibres and fusing to existing fibres, a process controlled by Myogenin. The balance of these decisions controls the ultimate size of the muscle. We want to understand the molecular mechanism(s) controlling in MSC behaviour during growth.

Project objectives will determine how MSCs respond to the growth stimulus and dissect genetically how the muscle senses physical force by:

a) performing single cell RNAseq to characterise stem cell diversity and obtain a molecular 'part-list'

and test the hypotheses that:

b) Myogenin regulation controls new fibre formation,
c) Myogenin cooperates with the cell cycle inhibitor Cdkn1c to regulate entry of MSCs into quiescence,
d) Diverse MSCs respond differently to quiescence/proliferation/differentiation signals.

New genome editing will be used to screen other candidate mechanosensitive cohort genes for roles in muscle growth.

Skills to be acquired. To analyse mutants the student will learn a series of approaches already used in the PI's laboratories: i) developmental genetics, ii) molecular biology, iii) 4D confocal microscopy, iv) stem cell biology, v) single cell RNAseq and other bioinformatic analysis.
By understanding force-dependent muscle growth, we aim to develop therapies for muscle diseases and to preserve muscle function in the elderly.

One representative publication from each co-supervisor:

Ganassi, M., Badodi, S., Ortuste Quiroga, P.O., Zammit, P.S., Hinits, Y. and S.M Hughes (2018) Myogenin promotes myocyte fusion to balance fibre number and size. ***Nat. Commun***. 9: 4232. www.nature.com/articles/s41467-018-06583-6/ PMID: 30315160

Kague, E., Hughes, S.M., Lawrence, E., Cross, S., Martin-Silverstone, E., Hammond*, C.L. and Y. Hinits*

(2019) Scleraxis genes are required for normal musculoskeletal development and for rib growth and mineralization in zebrafish. ***FASEB J.*** 33: 9116-9130 https://doi.org/10.1096/fj.201802654RR. PMID: 31100023

13.1 Novel regulators of the actin cytoskeleton in cytotoxic T cells.

Co Supervisor 1A: Robert Kochl
Research School/ Division or CAG: SIMS, Peter Gorer department of Immunobiology
Email: robert.koechl@kcl.ac.uk
Website:
https://www.kcl.ac.uk/lsm/research/divisions/diiid/departments/immunobiology/research/kochl/robert-köchl

Co Supervisor 1B: Claire Wells
Research School/ Division or CAG: Comprehensive Cancer Centre
Email: claire.wells@kcl.ac.uk
Website: https://www.kcl.ac.uk/people/claire-wells

Project Description:

Our group is interested in the understanding the signalling pathways that control the migration of cytotoxic T cells (CTLs) into infected tissues or into tumours and the subsequent killing of their target cells. We do this by a combination of in vitro biochemistry and cell biology approaches, including 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 tissues 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 to identify WNK1-dependent pathways and from year three onwards through pull downs and possibly also phosphoproteomics approaches to identify how WNK1 activates those pathways.

The work will be carried out in the Köchl lab in collaboration with Prof Claire Wells, an expert in studying pathways controlling actin dynamics.

One representative publication from each co-supervisor:

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.

PAK4 promotes kinase-independent stabilization of RhoU to modulate cell adhesion. Dart AE, Box GM, Court W, Gale ME, Brown JP, Pinder SE, Eccles SA, Wells CM. J Cell Biol. 2015 Nov 23;211(4):863-79.

16.1 Understanding how to manipulate the periosteal stem cell niche to tackle bone disease.

Co Supervisor 1A: Malcolm Logan
Research School/ Division or CAG: BMB, Randall Centre
Email: malcolm.logan@kcl.ac.uk
Website:
https://www.kcl.ac.uk/lsm/research/divisions/randall/research/sections/signalling/logan/loganmalcolm

Co Supervisor 1B: Karen Liu
Research School/ Division or CAG: Craniofacial and Regenerative Biology, FoDOCS
Email: karen.liu@kcl.ac.uk
Website: https://kclpure.kcl.ac.uk/portal/karen.liu.html

Project Description:

Understanding how to manipulate the periosteal stem cell niche to tackle bone disease

The periosteum is a thin layer of cells that covers the bone surface. Periosteal cells are essential for bone homeostasis, fracture repair and age-related loss of periosteum is associated with bone diseases such as osteoporosis. Despite the importance of the periosteum definitive identification of a periosteal stem cell population and methods to isolate and enrich these cells have not been established.

The project goal is to learn more about how periosteal stem cells contribute to the formation of new bone during bone homeostasis and fracture repair, how this is disrupted in bone disease and ageing and how these cells can be harnessed for therapeutic benefit.

Objectives:
Year 1-Using existing and new scRNAseq data, investigate the transcriptional 'signature' of periosteal stem cells and validate results in tissue sections and cell culture.
Year 2- Measure how distinct cell populations within the niche change under defined physiological conditions of clinical relevance, such as increasing age, load/stress using a combination of *in vivo* transgenic and *in vitro* assays
Year 3/4 Use genetic tools and agonists/antagonists to test the function of key signalling pathways.

Skills training:
The student will be trained in contemporary methods in stem cell, developmental and bone biology, the complementary use of genetically modified model organisms and *in vitro* culture methods, immunohistochemistry/histology and state of the art imaging/microscopy methods.

One representative publication from each co-supervisor:

Butterfield NC, Qian C, **Logan MPO** (**2017**) Pitx1 determines characteristic hindlimb morphologies in cartilage micromass culture. PLoS One 12(7):e0180453 doi: 10.1371/journal.pone.0180453. eCollection 2017 PMID:28746404

Gonzalez Malagon SG, Lopez Munoz AM, Doro D, Bolger T, Poon E, Tucker E, Adel Al-Lami H, Krause M, Phiel C, Chesler L and **Liu KJ.** GSK3 controls migration of the neural crest lineage. ***Nature Communications,*** 9, 116 **2018**. doi:10.1038/s41467-018-03512-5.

17.1 Regulation of physical forces and membrane remodelling to repair NERDIs and squeezed nuclei.

Co Supervisor 1A: Prof Juan Martin-Serrano
Research School/ Division or CAG: School of Immunology & Microbial Sciences, Infectious Diseases Department
Email: juan.martin_serrano@kcl.ac.uk
Website:
https://www.kcl.ac.uk/lsm/research/divisions/diiid/departments/infectious/research/Martin-Serrano/indexJD.aspx

Co Supervisor 1B: Dr Monica Agromayor
Research School/ Division or CAG: School of Immunology & Microbial Sciences, Infectious Diseases Department
Email: monica.agromayor@kcl.ac.uk
Website:
https://www.kcl.ac.uk/lsm/research/divisions/diiid/departments/infectious/research/agromayor/index.aspx

Project Description:

Compartmentalization of DNA in the nucleus is essential to avoid self-nucleic acid recognition and activation of innate immune responses. Cells migrating through constricted spaces can undergo *n*uclear *e*nvelope *r*upture *d*uring *i*nterphase (NERDI). These rupture events cause mislocalisation of the nuclear content and provide a mechanism by which self-DNA becomes exposed to the cytosol, thus inducing expression of interferon-stimulated genes (ISGs) via activation of the cGAS–STING pathway. NERDIs usually occur at sites where the nuclear membrane is weakened due to defects in lamina organisation and rupture is induced through contractile actin fibers that increase pressure on the nucleus via linker of nucleoskeleton and cytoskeleton (LINC) complexes. Importantly, recent studies have identified several cellular activities required for nuclear envelope resealing, including membrane remodelling by the endosomal sorting complex required for transport (ESCRT) machinery.

We have recently identified ESCRT-associated factors that regulate the mechanical properties of the nuclear envelope to allow repair after NERDI events. This project will build on these observations to explore how ESCRT-associated factors regulate the actin cytoskeleton to release physical tension at the nuclear envelope, thus facilitating nuclear envelope repair.

This project will also explore how ESCRT factors such as CHMP5 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.

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

"CC2D1B Coordinates ESCRT-III Activity during the Mitotic Reformation of the Nuclear Envelope." Ventimiglia *et al*. Dev Cell. 2018

"Knowing when to cut and run: mechanisms that control cytokinetic abscission." Agromayor & Martin-Serrano. Trends Cell Biol. 2013

18.1 How are immune cells directed towards sites of infection or inflammation?

Co Supervisor 1A: Prof Peter McNaughton
Research School/ Division or CAG: IoPPN, Wolfson CAR, FMedSci
Email: peter.mcnaughton@kcl.ac.uk
Website: www.kcl.ac.uk/mcnaughtonlab

Co Supervisor 1B: Dr Jon Robbins
Research School/ Division or CAG: Wolfson CARD
Email: jonathan.robbins@kcl.ac.uk
Website: https://www.kcl.ac.uk/ioppn/depts/wolfson/research/JRresearch/Jon-Robbins-Research.aspx

Project Description:

How are immune cells directed towards sites of infection or inflammation?
Neutrophils and macrophages (leukocytes) are key innate immune effector cells involved in early detection of danger signals that are released by tissue damage and pathogens. Leukocytes move towards these signals scavenge pathogens, initiate systemic immune responses and trigger injury resolution pathways. How is this vital cascade of events initiated? Recent evidence shows that hydrogen peroxide (H_2O_2) is released from damaged tissue by specific oxidases and leukocytes navigate towards the damage up this gradient of H_2O_2. How is H_2O_2 detected by leukocytes? Recent work in our lab shows that the ion channel TRPM2, which senses both elevated temperature and H_2O_2, is critical. TRPM2 is expressed in leukocytes and is calcium-permeable, so its activation promotes an influx of calcium into the leading edge of a leukocyte and thus steers it towards its target.

We have established an in vitro system to measure neutrophil/macrophage navigation and to measure intracellular calcium levels. We will investigate the role of TRPM2 in leukocyte motility, using a combination of molecular, bio-informatic, cellular and whole-animal techniques, and using human neutrophils/monocytes and mouse neutrophils/macrophages. We will also conduct *in vivo* studies with WT and TRPM2 KO mice in which we will monitor neutrophil/macrophage invasion into nerve following injury in vivo. How does sepsis change TRPM2 expression and leukocyte navigation, and can agonists/antagonists for TRPM2 be harnessed to improve leukocyte motility and thus aid in combatting life-threatening infection and inflammation?

Year 1: Role of TRPM2 and calcium signalling in neutrophil navigation

Year 2: Extend to macrophages, T cells.

Year 3: Immune cell invasion in animal models of injury and sepsis: role of TRPM2

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.

19.1 Investigating driver gene mutations in T-cell signalling pathways to identify therapeutic targets and genetic biomarkers for cutaneous T-cell lymphoma.

Co Supervisor 1A: Dr Tracey Mitchell
Research School/ Division or CAG: BMBS/St John's Institute of Dermatology/GRIDA
Email: tracey.mitchell@kcl.ac.uk
Website:
https://www.kcl.ac.uk/medicine/research/divisions/gmm/departments/dermatology/Groups/WhittakerLab/index.aspx
http://www.viapath.co.uk/our-people/dr-tracey-mitchell

Co Supervisor 1B: Prof Sean Whittaker
Research School/ Division or CAG: Genetics and Molecular Medicine/GRIDA
Email: sean.whittaker@kcl.ac.uk
Website:
https://www.kcl.ac.uk/medicine/research/divisions/gmm/departments/dermatology/Groups/WhittakerLab/index.aspx

Project Description:

Mapping the genomic landscape of tumour cells is a powerful approach to identify clinically actionable molecular targets and genetic biomarkers for diagnosis and patient stratification. Primary cutaneous T-Cell lymphoma (CTCL) is a heterogeneous malignancy of mature memory type, skin homing T-cells. There are no effective treatments for patients with advanced stage CTCL and as a consequence the survival rate is dismal (~3 years). Recent deep sequencing studies in CTCL have identified frequent gene mutations and copy number changes in key T-cell signalling genes including gain of function mutations that confer constitutive activation of PLC�1 and STAT3. The aims of this PhD are (i) to determine the downstream effects of *PLCG1* and *STAT3* gene mutations on T-cell survival pathways and (ii) to use integrated CTCL genomic data sets to identify biomarkers that can predict patient outcome. This approach is likely to have direct implications for CTCL diagnostics and therapeutics through selective targeting of patient-specific mutations.

Objectives:

Years 1-2: Functional analyses in cell lines and tumour cells to determine: the effect of *PLCG1* and *STAT3* gene mutations on T-cell survival pathways; evidence for molecular crosstalk between PLC�1 and STAT3; the effect of mutation on PLC�1 and STAT3 cellular localisation.

Years 2-3: Design of diagnostic/prognostic targeted capture gene panel using integrated CTCL genomic datasets. Validation of the panel on tumour DNA from our Biobank. Univariate and multivariate analyses to determine the clinical efficacy of the panel.

Skills training:

'Wet lab' techniques: cell culture; FLOW; functional assays (including transformation, proliferation, apoptosis); RNA-seq.

Bioinformatics: NGS data analysis (targeted capture and RNAseq); gene set enrichment analysis; statistical packages.

One representative publication from each co-supervisor:

Woollard WJ, Pullabhatla V, Lorenc A, Patel VM, Butler RM, Bayega A, Begum N, Bakr F, Dedhia K, Fisher J, Aguilar-Duran S, Flanagan C, Ghasemi AA, Hoffmann RM,Castillo-Mosquera N, Nuttall EA, Paul A, Roberts CA, Solomonidis EG, Tarrant R,Yoxall A, Beyers CZ, Ferreira S, Tosi I, Simpson MA, de Rinaldis E, **Mitchell TJ**, **Whittaker SJ**. Candidate driver genes involved in genome maintenance and DNA repair in Sézary syndrome. Blood. 2016 Jun 30;127(26):3387-97. doi:10.1182/blood-2016-02-699843.

Patel VM, Flanagan CE, Martins M, Jones CL, Butler RM, Woollard WJ, Bakr FS, Yoxall A, Begum N, Katan M, **Whittaker SJ, Mitchell TJ**. Frequent and persistent PLCG1 mutations in Sézary cells directly enhance PLCγ1 activity and stimulate NFκB, AP-1 and NFAT signalling. J Invest Dermatol. 2019 Jul 31. pii: S0022-202X(19)32679-X. doi: 10.1016/j.jid.2019.07.693. [Epub ahead of print] PubMed PMID: 31376383.

20.1 Explaining the sexual dimorphism in Lupus through genetics.

Co Supervisor 1A: Dr David Morris
Research School/ Division or CAG: School of Medicine
Email: david.l.morris@kcl.ac.uk
Website:
https://www.kcl.ac.uk/lsm/research/divisions/gmm/departments/mmg/researchgroups/VyseLab/dmorris.aspx

Co Supervisor 1B: Prof Timothy Vyse
Research School/ Division or CAG: School of Medicine
Email: timothy.vyse@kcl.ac.uk
Website:
https://www.kcl.ac.uk/lsm/research/divisions/gmm/departments/mmg/researchgroups/VyseLab/index.aspx

Project Description:

Systemic Lupus Erythematosus (SLE) is an autoimmune disease that affects millions of people worldwide. Strikingly 9/10 SLE cases are women, yet little is understood as to why. Hormonal and environmental factors are believed to be partly responsible, and while there is strong evidence for a genetic basis for this dimorphism there is still a gulf in our understating. There are many avenues ripe for investigation and, with the advent of new technologies and a large amount of data available, a thorough study of the differences in genetics between the sexes is warranted. Current interests include the overlap between SLE associated genetic loci and genes showing sex differences in expression, genetic associations on the X chromosome and the cellular origins of effects.

This PhD will investigate all forms of genetic variation between the sexes that are informative of the sexual dimorphism of SLE, applying cutting edge statistical techniques on the richest data on SLE in the world. A background in statistics is not required but an interest in analyses is important. The student will learn the statistical language R and run modern genetic analyses software to a high standard making them very competitive in the current research environment. The study will use the largest collection of SLE genetic data in the world together with gene expression data from several sources including single cell data and very novel data on ChrX inactivation in SLE patients as part of the Open Targets project (https://www.opentargets.org/) in collaboration with the sanger institute.

Year 1:

There are multiple as yet undetected SLE associated loci on Chromosome X

Aim: Conduct a meta-analysis using three published European GWAS and two published Chinese GWAS as discovery together with a further new European GWAS and a new Chinese GWAS to test the X chromosome for association with SLE. Test for difference in association between male and females.

Year 2:

The genetic associations on chromosome X will be explained by functional data and likely effecting disease though altered gene expression.

Aim: Annotate known and novel associations using public ally available data on gene expression data and genomic structure together with new data on gene expression generated in the lab.

Year 3:

The genetics of SLE on chromosome X effects sex specific biological pathways.

Aim: Perform pathway analysis using data generated form years 1-2 to determine the causal link between genetics and sex specific risk for disease. The student will also look at the overlap between X chromosome genetic susceptibility genes and genes that show loss of X-Chromosome Inactivation, using novel data form the lab, testing the hypothesis that genetic factors and X chromosome-related aberrant regulation act in a complementary manner to increase disease risk.

One representative publication from each co-supervisor:

Morris DL, Sheng Y, Zhang Y, Wang Y-F, Zhu Z, Tombleson P, Chen L, Graham D S-C, Bentham J, Chen R, Zuo X, Wang T, Wen L, Yang C, Liu L, Yang L, Li F, Huang Y, Yin X, Yang S, Rönnblom L, Fürnrohr BG, Voll RE, Schett G, Costedoat–Chalumeau N, Gaffney PM, Lau YL, Zhang X, Yang W, Cui Y, **Vyse TJ.** (2016). Genome-wide association meta-analysis in Chinese and European individuals identifies ten new loci associated with systemic lupus erythematosus. *Nature Genetics*. doi: **10.1038/ng.3603. Aug; 48(8): 940-946**
enhance PLCγ1 activity and stimulate NFκB, AP-1 and NFAT signalling. J Invest Dermatol. 2019 Jul 31. pii: S0022-202X(19)32679-X. doi: 10.1016/j.jid.2019.07.693. [Epub ahead of print] PubMed PMID: 31376383.

Bentham J, **Morris DL**, Graham DSC, Pinder CL, Tombleson P, Behrens TW, Martín J, Fairfax BP, Knight JC, Chen L, Replogle J, Syvänen A-C, Rönnblom L, Graham RR, Wither JE, Rioux JD, Alarcón-Riquelme ME & **Vyse TJ.** (2015). Genetic association analyses implicate aberrant regulation of innate and adaptive immunity genes genes in the pathogenesis of systemic lupus erythematosus *Nature Genetics*. doi:10.1038/ng.3434 Dec; 47(12) 1457-64

21.1The candidalysin interactome: Characterisation of novel interactions between human epithelial cells and the fungal peptide toxin candidalysin.

Co Supervisor 1A: Julian Naglik
Research School/ Division or CAG: Centre for Host-Microbiome Interactions
Email: julian.naglik@kcl.ac.uk
Website: https://kclpure.kcl.ac.uk/portal/julian.naglik.html

Co Supervisor 1B: Dr Jonathan Richardson
Research School/ Division or CAG: Centre for Host-Microbiome Interactions
Email: jonathan.richardson@kcl.ac.uk
Website: https://kclpure.kcl.ac.uk/portal/jonathan.richardson.html

Project Description:

Epithelial cells are typically the first point of contact between the host and commensal/pathogenic microbes. Mucosal barriers have evolved to initiate a range of protective immune responses designed to combat infecting pathogens. During mucosal infection, the fungal pathogen *Candida albicans* secretes candidalysin, a peptide toxin that causes epithelial damage and triggers EGFR-mediated immune responses through MAPK signalling, identifying the MAPK pathway as an important component of the host response to *C. albicans* infection. However, our understanding of epithelial signalling responses to candidalysin is currently incomplete.

This project will combine CRISPR screening technologies together with *in vitro* and *in vivo* models to identify and characterise novel epithelial responses to candidalysin. We anticipate that signalling pathways involved in immune responses, intracellular stress and damage repair will be identified.

Year 1 will use established CRISPR technologies to identify novel epithelial signalling pathways involved in the response to candidalysin.

In year 2, secondary validation and mechanisms of pathway activation will be investigated *in vitro*.

In year 3, analysis of pathway activation *in vivo* will be performed together with the use of established murine gene knock out models (for example: c-Fos, MKP1, IL-1R, EGFR, Flotillin1/2) to elucidate precise mechanisms of host response during *C. albicans* infection.

Full training in all relevant technologies will be provided.

This challenging project combines host-pathogen interaction, mammalian/fungal biology, CRISPR technologies/molecular biology, and immunology together with *in vitro*/*in vivo* infection models and will encourage student growth and development in an enthusiastic and supportive research environment.

One representative publication from each co-supervisor:

Moyes DL, Wilson D, Richardson JP, Mogavero S, Tang SX, Wernecke J, Höfs S, Gratacap RL, 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, Luo T, Krüger T, Kniemeyer 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.

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, Gaffen SL, Hube B and Naglik JR **(2018)**. Processing of *Candida albicans* Ece1p is critical for Candidalysin maturation and fungal virulence. **mBIO**. DOI: 10.1128/mBio.02178-17.

22.1 Identification of host cell proteins interacting with Hepatitis Delta Virus Antigen that regulate virus replication.

Co Supervisor 1A: Prof Stuart Neil
Research School/ Division or CAG: SIMS
Email: stuart.neil@kcl.ac.uk
Website: https://kclpure.kcl.ac.uk/portal/stuart.neil.html

Co Supervisor 1B: Dr Ivana Carey
Research School/ Division or CAG: SIMS
Email: ivana.kraslova@kcl.ac.uk
Website: https://kclpure.kcl.ac.uk/portal/ivana.kraslova.html

Project Description:

Hepatitis Delta Virus (HDV) is the smallest known human virus with a circular, self-complementary genome that encodes a single gene from which are expressed long and short isoforms of the Delta Antigen (L-HDag and S-HDag). HDV replication is entirely dependent on Hepatitis B Virus (HBV) and is transmitted between liver cells in HBV-like particles. Around 5% of HBV chronically infected individuals (15-20 million worldwide) are co-infected with HDV, which greatly enhances liver pathology and the progression to cirrhosis and hepatocellular carcinoma. There are at least 8 genotypes of HDV with variable prevalence across the world. Interestingly, some genotypes appear to more easily treated with pegylated type 1 interferon (IFN) than others. While it is known that S-HDag is essential for genome replication and L-HDag for packaging into HBV virions, much of the underlying molecular and cellular biology of HDV replication remains to be discovered.

The student will draw on the experience of the Viral Hepatitis unit at King's College Hospital in collaboration in the Neil lab to use comparative proteomics to identify cellular proteins that interact with representative examples of L and S-HDag of major HDV genotypes from human hepatoma cells. They will establish HDV/HBV replicon systems to characterise the relevance of these identified factors using CRISPR/Cas9 gene knockouts to assay the role of these factors on HDV (or HBV) genome replication, viral assembly and the sensitivity to type I IFNs. Of particular interest will be host cell factors that are "druggable" and those that differentially interact with HDags of major genotypes. Findings will then be applied to the study of relevant patient samples bio-banked in the Viral Hepatitis unit.

Techniques: Tandem Mass-tagging and SILAC-based proteomics, CRISPR/Cas9-based gene knockouts, culture and propagation of human hepatitis viruses, protein biochemistry, a wide range of molecular biology, fluorescence microscopy.

Rotational Yr: clone and express HDags in human hepatoma cells; characterise the interaction of HDags with MOV10

Yr 1: Proteomic screens of interacting factors +/- HDV genome +/- IFN; establishment of viral replication systems.
Yr 2-3: Characterization of identified factors

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

The role of anti-HBs in hepatitis B reactivation during direct-acting antiviral therapy for chronic hepatitis C.

Spaan M, Bruce M, Agarwal K, Carey I.
Antivir Ther. 2018;23(6):539-542. doi: 10.3851/IMP3259.
PMID: 30309997

Resistance of Transmitted Founder HIV-1 to IFITM-Mediated Restriction.
Foster TL, Wilson H, Iyer SS, Coss K, Doores K, Smith S, Kellam P, Finzi A, Borrow P, Hahn BH, Neil SJD.
Cell Host Microbe. 2016 Oct 12;20(4):429-442. doi: 10.1016/j.chom.2016.08.006. Epub 2016 Sep 15.

PMID: 27640936

23.1 Immune-gut interactions in food allergy and oral tolerance

Co Supervisor 1A: Alexandra F. Santos
Research School/ Division or CAG: Life Course Sciences
Email: alexandra.santos@kcl.ac.uk
Website: https://www.kcl.ac.uk/research/santos-group

Co Supervisor 1B: Joana F Neves
Research School/ Division or CAG: Centre for Host-Microbiome Interactions
Email: joana.pereira_das_neves@kcl.ac.uk
Website: https://www.kcl.ac.uk/people/joana-f-neves

Project Description:

Food allergy affects 7% of children in Western countries and can cause severe allergic reactions and fatal anaphylaxis. There is currently no curative treatment for food allergy. Understanding the immune mechanisms underlying food allergy and oral tolerance could enable us to identify novel targets for definitive treatment.

Gut tissue from the small intestine is mostly inaccessible in humans. Gut organoids derived from human stem cells have been developed at KCL and will be used as a surrogate for human intestine to study, for the first time, the interactions between gut and immune cells during the establishment of normal oral tolerance or aberrant allergic response to foods. Using this innovative intestinal organoid approach samples from allergic and non-allergic children being assessed for cow's milk, egg, sesame, cashew or peanut allergies, as part of ongoing clinical studies, will be studied through a variety of approaches.

The successful student will acquire theoretical and practical skills in cell biology (cell isolation and culture, generation of gut organoids, flow cytometry), functional genomics (RNAseq), and molecular immunology (RT-PCR, siRNA, lentiviral overexpression, CRISPR) as well as translational research skills in allergy and clinical immunology and bioinformatics.

Objectives for each year:

- Year 1: Co-culture of gut organoids with patients' immune cells; Flow cytometry and Luminex assays to study the immune interactions.
- Year 2: Single cell RNA sequencing.
- Year 3: Validation of findings in human gut biopsies.
- Year 4: Statistical analyses and write up.

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

Hemmings O, Du Toit G, Radulovic S, Lack G, Santos AF. Ara h 2 is the dominant peanut allergen despite similarities with Ara h 6. J Allergy Clin Immunol 2020;146(3):621-630.e5

Jowett GM, Norman MDA, Yu TTL, Arévalo PR, Hoogland D, Lust S, Read E, Hamrud E, Walters NJ, Niazi U, Chung MWH, Marciano D, Omer OS, Zabinski T, Danovi D, Lord GM, Hilborn J, Evans ND, Dreiss C, Bozec L, Oommene OP, Lorenz C, Silva RMP, Neves JF, Gentleman E. ILC1 drives intestinal and matrix remodelling. Nature Materials 2020

24.1 Roles of the interferon stimulated gene NCOA7 in antibacterial immunity.

Co Supervisor 1A: Dr Charlotte Odendall
Research School/ Division or CAG: SIMS, Department of Infectious Diseases
Email: charlotte.odendall@kcl.ac.uk
Website:
https://www.kcl.ac.uk/lsm/research/divisions/diiid/departments/infectious/research/odendall/index

Co Supervisor 1B: Prof Michael Malim
Research School/ Division or CAG: School of Immunology and Microbial Sciences
Email: michael.malim@kcl.ac.uk
Website: http://www.kcl.ac.uk/malim

Project Description:

Type I interferons (IFNs) are cytokines produced following recognition of pathogens by the immune system. IFNs induce a large family of proteins termed IFN-stimulated genes (ISGs) that collectively combat infection by establishing an anti-microbial state. One particular ISG, **NCOA7,** was recently shown to interact with vacuolar ATPase (v-ATPase), a proton pump that acidifies endosomes and promotes the maturation of phagosomes. NCOA7 alone blocks infection with a number of viruses. Although type I IFNs are best known for their antiviral properties, they also have antibacterial functions. We have found that type I IFN treatment inhibits intracellular replication of bacteria such as *Salmonella*, but the effector ISGs are not known. Since v-ATPase has a central role in the clearance of bacteria phagocytosed by immune cells, we hypothesise that NCOA7 contributes to the acidification of bacteria-containing endosomes and phagosomes, thus controlling the fate of intracellular bacteria. The aims of this project are to:

1- Define if NCOA7 enhances killing of pathogenic/ non-pathogenic bacteria in phagosomes (Year 1)
2- Characterise the molecular mechanisms that drive NCOA7 antiviral and antibacterial activities (Year 1 and 2)

3- Study NCOA7 function in well-established mouse models of infection (Year 2 and 3)
Wild-type (WT) or NCOA7 knockout (KO) macrophages will be purified from mouse bone marrow. After IFN treatment, they will be infected with bacterial strains. These will include non-pathogenic bacteria normally killed by macrophages, and pathogenic bacteria such as *Salmonella* that thrive within phagosomes. Bacterial survival will be quantified by plating and enumeration of live colony forming units (CFUs). In follow up assays, phagosome function and lysosomal protease activity will be quantified using biochemical and microscopy-based assays. *In vivo* studies will be carried out in a mouse model of systemic *Salmonella* infection, with bacterial growth in spleen and liver quantified by CFU plating.

Techniques used will therefore include:

- Molecular Biology and Microbiology

- Biochemistry
- Tissue culture
- Flow cytometry and microscopy
- *In vitro* replication and survival assays
- Mouse model of *Salmonella* infection

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

1- **Odendall**, C., Voak, A.A., and Kagan, J.C. (2017). Type III IFNs Are Commonly Induced by Bacteria-Sensing TLRs and Reinforce Epithelial Barriers during Infection. The Journal of Immunology *199*, 3270–3279.

2- Doyle, T., Moncorgé, O., Bonaventure, B., Pollpeter, D., Lussignol, M., Tauziet, M., Apolonia, L., Catanese, M.-T., Goujon, C., and **Malim**, M.H. (2018). The interferon-inducible isoform of NCOA7 inhibits endosome-mediated viral entry. Nature Microbiology *3*, 1369–1376.

25.1 Understanding chromatin remodelling at the nuclear periphery.

Co Supervisor 1A: Prof Snezhana Oliferenko
Research School/ Division or CAG: Basic and Medical Biosciences / Randall Centre for Cell and Molecular Biophysics
Email: snezhana.oliferenko@kcl.ac.uk
Website: https://www.kcl.ac.uk/people/snezhana-oliferenko

Co Supervisor 1B: Dr Jeremy Carlton
Research School/ Division or CAG: School of Cancer and Pharmaceutical Sciences
Email: jeremy.carlton@kcl.ac.uk
Website: https://www.kcl.ac.uk/lsm/research/divisions/cancer/research/groups/mtcd.aspx

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. 2019. ESCRT-III/Vps4 controls heterochromatin-nuclear envelope attachments. BioRxiv doi: https://doi.org/10.1101/579805. In revision for *Developmental Cell*.

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

26.1 Defining the role of mechanical forces in fibrosis.

Co Supervisor 1A: Prof Maddy Parsons
Research School/ Division or CAG: BMBS/ Randall
Email: maddy.parsons@kcl.ac.uk
Website: https://www.kcl.ac.uk/research/profile/the-parsons-group

Co Supervisor 1B: Dr Susan Cox
Research School/ Division or CAG: BMBS/Randall
Email: susan.cox@kcl.ac.uk
Website: : https://www.kcl.ac.uk/lsm/research/divisions/randall/people/profiles/coxsusan

Project Description:

The physical and mechanical properties of the tissue environment strongly influence cell phenotype and behaviour. Controlling the local physical environment of cells plays an essential role in developmental processes and tissue homeostasis; this balance is also disrupted during progression of many diseases including fibrosis and cancer. Cells do not simply respond to their surrounding extracellular matrix (ECM) environment, they create and remodel it. This potentially operates through a feedback loop, whereby cells respond and react to external mechanical cues leading to secretion of new ECM proteins. This in turn alters the stiffness of the environment, changing the cell phenotype and behaviour. Despite their importance, the feedback mechanisms between the microenvironment, mechano-sensing response of the cell and deposition of new matrix remain poorly understood. The aim of this project is to define this biophysical feedback that controls matrix homeostasis. The key goals are:

1. **Determine the contribution of tissue mechanics to de novo ECM production/destruction (yrs1/2) –** using 3D in vitro models of differing ECM stiffness, RNA sequencing, proteomics and advanced live imaging will be used to define the key proteins in fibroblasts that alter expression during the pro-fibrotic switch

2. **Define force-transduction pathways controlling ECM biosynthesis (yrs 2/3) -** live cell fluorescence imaging in mechanically-tuned ECM scaffolds will be used to quantify F-actin dynamics, membrane/focal adhesion tension. Novel biosensors that report on cytoskeletal regulators that transduce force signals will be analysed. Key regulatory proteins identified in Aim1 will be knocked out by CRISPR/Cas9 and the effects on ECM stiffness quantified by atomic force microscopy.

Data arising from this study will extend our understanding of mechanical feedback to physiological settings and through systematic real-time analysis, will determine the key triggers controlling tissue homeostasis and fibrosis.

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

Fascin Regulates Nuclear Movement and Deformation in Migrating Cells
Jayo, A., Malboubi, M., Antoku, S., Chang, W., Ortiz-Zapater, E., Groen, C., Pfisterer, K., Tootle, T., Charras, G., Gundersen, GG. & Parsons, M., 22 Aug 2016, Developmental Cell. 38, 4, p. 371-383

Artifact-free high-density localization microscopy analysis

Marsh, R. J., Pfisterer, K., Bennett, P., Hirvonen, L. M., Gautel, M., Jones, G. E. & Cox, S., Sep 2018, Nature Methods. 15, p. 689-692

27.1 Platelets and allergen sensitization: A critical interface between trained innate immunity and the adaptive immune response.

Co Supervisor 1A: Dr Simon Pitchford
Research School/ Division or CAG: Cancer and Pharmaceutical Sciences CAG: Pharmaceutical Sciences
Email: simon.pitchford@kcl.ac.uk
Website: https://www.kcl.ac.uk/people/simon-pitchford

Co Supervisor 1B: Prof Clive Page
Research School/ Division or CAG: Cancer and Pharmaceutical Sciences CAG: Pharmaceutical Sciences
Email: clive.page@kcl.ac.uk
Website: https://www.kcl.ac.uk/people/clive-page

Project Description:

Background. Platelets have been recognized for some time to act as inflammatory cells in the defence of the body against infection, performing many functions normally associated with leukocytes. These roles are distinct from platelet function during haemostasis. Interestingly, platelets act as a 'bridge' between the innate and adaptive immune response. In particular, platelets are activated in patients with asthma, and are responsible for the misdirected inflammatory response. Recently, we reported that platelets migrate into lung tissue upon allergen sensitization and challenge and associate with lung dendritic cells, and that temporary platelet depletion at the time of initial allergen sensitization resulted in reduced inflammatory responses upon subsequent, secondary allergen exposure. We outline a PhD programme to investigate how the process of antigen sensitization affects platelet activity and the development of immune memory. Future impact might lead to alternative strategies for 'disease modifying' therapies of allergic disease or infections.

Details of Techniques: *In vivo* skills pertinent to murine models of allergic lung inflammation: allergen sensitization and exposure procedures, cell and tissue harvesting and purification, immunohistochemistry. An exciting research avenue is advanced real time imaging techniques to record, for example, antigen presenting cell and platelet localization in mice. *In vitro* functional assays to elucidate platelet activation, function, and interactions with innate immune cells (e.g. flow cytometry, chemotaxis).

Objectives:
Year 1. How does antigen exposure modulate platelet production and phenotype?

Year 2. How do platelets stimulate innate immune cells, their tissue recruitment and transit?

Year 3-4. How do platelets modulate antigen sensitization and recognition?

One representative publication from each co-supervisor:

Pitchford: Amison RT et al. Platelets play a central role in sensitisation to allergen. Am J Respir Cell Mol Biol. 2018. 59: 96-103.

Page: Idzko M, Pitchford S, Page C. Role of Platelets in allergic airway inflammation. *J Allergy Clin Immunol.* 2015;135:1416-1423.

28.1 Generation of tissue specific CAR-Tregs to modulate liver inflammatory and promote immune tolerance.

Co Supervisor 1A: Prof Alberto Sanchez-Fueyo
Research School/ Division or CAG: Department of Inflammation Biology / Institute of Liver Studies / School of Immunology and Microbial Sciences / Faculty of Life Sciences & Medicine
Email: sanchez_fueyo@kcl.ac.uk
Website: https://kclpure.kcl.ac.uk/portal/sanchez_fueyo.html

Co Supervisor 1B: Dr Gilbert Fruhwirth
Research School/ Division or CAG: School of Biomedical Engineering & Imaging Sciences
Email: gilbert.fruhwirth@kcl.ac.uk
Website: https://kclpure.kcl.ac.uk/portal/en/persons/gilbert-fruhwirth(e089cca1-3041-4cae-92ed-c401bc7a2ba5)/biography.html

Project Description:

The burden of liver disease is increasing in the UK, becoming the third most common cause of premature death. End-stage liver diseases are characterised by dysregulated inflammation and inefficient hepatocyte regeneration. Regulatory T cells (Tregs) play a key role in the control of most inflammatory diseases. In addition, Tregs can elicit tissue repair and pro-regenerative programmes following muscle or lung injury. Whether similar effects can be observed following liver injury remains to be investigated. Conferring Tregs specificity against tissue-restricted antigens provides a means to compartimentalize their suppressive and tissue repair properties to specific diseased organs.

Cellular therapy with chimeric antigen receptor (CAR)-redirected cytotoxic T cells has shown impressive efficacy in the treatment of hematologic malignancies. CAR-based strategies can be successfully applied to Tregs as well and hold promise as a novel immunotherapeutic strategy to treat autoimmunity, transplantation and organ failure. We propose to design a liver-specific CAR Treg to explore the extent to which Tregs can restore the intrahepatic immune balance during chronic inflammation and enhance tissue regeneration.

Year 1: Design and construction of CAR-Tregs. Implementation of *in vitro* models to assess Treg specificity and effects on primary liver cells.
Year 2: *In vivo* validation in murine models of inflammatory liver disease. Cell tracking experiments.
Years 3-4: Studying the mechanisms underlying intrahepatic immunoregulation and tissue repair mediated by CAR-Tregs. Data analysis and thesis write-up.

One representative publication from each co-supervisor:

- Whitehouse G, Gray E, Mastoridis S, Merritt E, Kodela E, Yang JHM, Danger R, Mairal M, Christakoudi S, Lozano JJ, Macdougall IC, Tree T, **Sanchez-Fueyo A**, Martinez-Llordella M.
IL-2 therapy restores regulatory T-cell dysfunction induced by calcineurin inhibitors
PNAS. 2017; 114(27):7083-7088

- Boardman DA, Philippeos C, **Fruhwirth GO**, Ibrahim MA, Hannen RF, Cooper D, Marelli-Berg FM, Watt FM, Lechler RI, Maher J, Smyth LA, Lombardi G.

Expression of a Chimeric Antigen Receptor Specific for Donor HLA Class I Enhances the Potency of Human Regulatory T Cells in Preventing Human Skin Transplant Rejection
Am J Transplant. 2017 Apr;17(4):931-943

29.1 The influence of co-stimulatory domains on the metabolic regulation of the chimeric antigen receptor (CAR) T cell function.

Co Supervisor 1A: Anna Schurich
Research School/ Division or CAG: FoLSM, Department of Infectious Diseases
Email: anna.schurich@kcl.ac.uk
Website: https://www.kcl.ac.uk/people/anna-schurich

Co Supervisor 1B: John Maher
Research School/ Division or CAG: Cancer and Pharmaceutical Sciences/ Cancer
Email: john.maher@kcl.ac.uk
Website: https://kclpure.kcl.ac.uk/portal/john.maher.html

Project Description:

T-cells are a vital component of the immune system and promoting/engineering T-cell responses is now at the forefront of cancer immuno-therapies. The ability of a T-cell to display full effector functions (e.g. proliferation, cytotoxicity) crucially depends on optimal cellular energy utilisation and the availability of nutrients in the environment. Cancer cells in solid tumours can counteract this immune response by manipulating the availability of nutrients in the tumour microenvironment (TME), leading to treatment failure. We developed chimeric antigen receptor (CAR) T-cells with novel co-stimulatory domains to specifically target solid tumours. As a PhD student with us you will investigate the influence of co-stimulation on the cellular metabolism, to learn how we can manipulate this pathway to increase the cells longevity and functionality in the TME. In the first year you will gain in depth knowledge in human T-cell/cancer biology and immunometabolism, learning how to produce, culture and metabolically challenge CAR T-cells and receive formal training in advanced flowcytometry by the BRC flow core and PI. In the second phase you will use your knowledge to modify your CAR platform, thus you will gain an understanding of how to genetically engineer T-cells. In the third phase you will validate your optimised construct/s and test the most promising candidate in a pilot in-vivo model study. This project is highly translational and your research has the potential to contribute to clinical trials. AS's and JM's groups are working closely together and we are located in the same building, facilitating exchange.

One representative publication from each co-supervisor:

Schurich A, Pallett LJ, Jajbhay D, Wijngaarden J, Otano I, Gill US et al. Distinct Metabolic Requirements of Exhausted and Functional Virus-Specific CD8 T Cells in the Same Host. Cell Rep 2016; 16(5): 1243-1252. doi: 10.1016/j.celrep.2016.06.078

Whilding LM, Halim L, Draper B, Parente-Pereira AC, Zabinski T, Davies DM, Maher J (2019) CAR T-cells targeting the integrin avb6 and co-expressing the chemokine receptor CXCR2 demonstrate enhanced homing and efficacy against several solid malignancies. Cancers. 11(5) pii: E674. doi: 10.3390/cancers11050674.

30.1 Integrative personalised analysis of immunome, microbiome and metabolome in cancer patients.

Co Supervisor 1A: Dr Saeed Shoaie
Research School/ Division or CAG: Centre for Host-Microbiome Interactions - FoDOC
Email: saeed.shoaie@kcl.ac.uk
Website: https://www.kcl.ac.uk/people/saeed-shoaie-1

Co Supervisor 1B: Dr Shahram Kordasti
Research School/ Division or CAG: Cancer & Pharmaceutical Sciences, Comprehensive Cancer Centre, FOLSM
Email: shahram.kordasti@kcl.ac.uk
Website: https://www.kcl.ac.uk/people/shahram-kordasti

Project Description:

Myeloid malignancies such as Myelodysplatic syndrome (MDS) and subsequent acute myeloid leukaemia (AML) incidence is set to rise substantially due to population aging, with exponential increases in health care costs. Current prognostic scoring system is an ineffective predictor of response to therapies in these patients.
The hypothesis of this project is that a multi-omics approach and integration of clinical and OMICs data could better stratify these patients for response to therapy and better predict disease progression.

During the rotation project, the student will explore this hypothesis by associated microbiome and immune responses together with functional and clinical data. This project will provide the student with the cutting-edge systems analysis tools to perform and integrate multi-omics data to address an important clinical question.

First Year – Microbiome data generation
The student will learn and analyse relevant gut and oral microbiome data on the haematological malignancies' cohorts. Students will learn how to deal with shotgun metagenomics data, using the latest tools and approaches such as constructing specific gut and oral microbiome catalogues.

Second Year – Immunome data generation
The student will be trained and analysed deep phenotyping of immune and malignant cells, using a combination of multi-parameter mass cytometry (CyTOF), scRNA sequencing as well as metabolomics as part of 'immunome' signatures.

Third year – Data integration
Genome-Scale Model (GEM) will be used to integrate different data OMICs data. This will help us to better stratify patients in terms of overall immune response and the potential effect of this response on survival and disease progression.

One representative publication from each co-supervisor:

Shoaie S, Ghaffari P, Kovatcheva-Datchary P, Mardinoglu A, et al
Quantifying Diet-Induced Metabolic Changes of the Human Gut Microbiome.
Cell Metab. 2015 Aug 4;22(2):320-31

Kordasti S, Costantini B, Seidl T, Perez Abellan P, et al
Deep phenotyping of Tregs identifies an immune signature for idiopathic aplastic anemia and predicts response to treatment.
Blood. 2016 Sep 1;128(9):1193-205.

31.1 Regulation of chromosome X-inactivation in ageing and auto-immune disease.

Co Supervisor 1A: Kerrin Small
Research School/ Division or CAG: School of Life Course Sciences
Email: kerrin.small@kcl.ac.uk
Website:
http://www.kcl.ac.uk/lsm/research/divisions/gmm/departments/twin/research/small/index.aspx

Co Supervisor 1B: Jordana Bell
Research School/ Division or CAG: School of Life Course Sciences
Email: jordana.bell@kcl.ac.uk
Website: http://epigen.kcl.ac.uk/

Project Description:

To maintain a consistent level of gene expression between the sexes, females silence or inactivate one X chromosome in each cell. The inactivated X is randomly selected, but as women age, one of the two X's starts to predominate in some tissues. Age-related skewed X-inactivation is heritable in twin studies, but it is not known how genetics or which environmental factors impact age-related skewing. Skewed X-inactivation is a crucial risk factor for several diseases in females, including auto-immune and age-related diseases.

This project will explore the interplay between skewed X-inactivation and genetics, age, ageing-related disease, and lifestyle factors in a unique dataset of 4000 deeply phenotyped female twins from the TwinsUK cohort (twinsuk.ac.uk). Further, in humans, 12-20% of genes on the inactive X are expressed, they escape X-inactivation. Escape varies across individuals and tissues in terms of presence and strength and contributes to risk of auto-immune disease and cancer. This project will investigate the role of genetics, epigenetics and environmental factors in regulating escape from X-inactivation using deep RNA-sequencing in purified cells from selected twin pairs and large 'omic datasets, and investigate how escape influences auto-immune disease-associated genes.

Objectives:
Year 1 Genetics and epigenetics of age-related skewed X-inactivation
Year 2 Cell-type and individual variability in escape from X-inactivation
Year 3 Integration of auto-immune disease risk genes and chromosome X regulation

The project will provide skills training in genetic and genomic analyses, DNA and RNA profiling, RNA-seq analysis, epigenomics and twin modelling.

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

Zito A, Davies MN, Tsai PC, Roberts S, Nardone S, Bell JT, Wong CY, Small KS Heritability of skewed X-inactivation in female twins is tissue-specific and dependent on age *bioRxiv*
doi:https://doi.org/10.1101/593251

Luijk R, Wu H, Ward-Caviness CK, Hannon E, Carnero-Montoro E, Min JL, Mandaviya P, Müller-Nurasyid M, Mei H, van der Maarel SM; BIOS Consortium, Relton C, Mill J, Waldenberger M, Bell JT, Jansen R, Zhernakova A, Franke L, 't Hoen PAC, Boomsma DI, van Duijn CM, van Greevenbroek MMJ, Veldink JH, Wijmenga C, van Meurs J, Daxinger L, Slagboom PE, van Zwet EW, Heijmans BT. Autosomal genetic variation is associated with DNA methylation in regions variably escaping X-chromosome inactivation. Nat Commun. 2018 Sep 14;9(1):3738. doi: 10.1038/s41467-018-05714-3.

32.1 Studying the molecular control of the pancreatic mesenchyme on beta-cell differentiation.

Co Supervisor 1A: Francesca M. Spagnoli
Research School/ Division or CAG: School of Basic and Medical Biosciences, CSCRM
Email: francesca.spagnoli@kcl.ac.uk
Website: https://www.kcl.ac.uk/lsm/research/divisions/gmm/departments/stemcells/index.aspx

Co Supervisor 1B: Alessandra Vigilante
Research School/ Division or CAG: School of Basic and Medical Biosciences, CSCRM
Email: alessandra.vigilante@kcl.ac.uk
Website: https://www.kcl.ac.uk/lsm/research/divisions/gmm/departments/stemcells/index.aspx

Project Description:

Cell identity is imparted by temporal and spatial integration of extrinsic signals from the microenvironment and intrinsic determinants. Pancreatic beta-cell differentiation and functionality is dependent on surrounding mesenchymal cells. Despite the relevance of such cell-cell interaction(s), little is known about the cellular composition of the pancreatic mesenchyme and how it governs cell differentiation. *This knowledge will be valuable for developing cell-replacement therapies to treat diabetes, which remains incurable.*

The Spagnoli laboratory has recently identified a specialized mesenchymal population in the embryonic pancreas, which promotes pancreatic beta-cell differentiation (manuscript under revision). These findings defined a mesenchymal “niche” producing pro-endocrine instructive signals under the control of the transcription factor *Pbx1*. Previous RNASeq analyses identified PBX1-downstream targets controlling beta-cell differentiation in the pancreas. *This PhD project will build on these findings and investigate the epithelium-mesenchyme crosstalk in pancreatic beta-cell differentiation with the final goal to improve the functional properties of stem-cell-derived beta-cells for therapeutic purposes.*

Aim 1 (Years 1/2). To elucidate the mechanism of action of PBX1 within the pancreatic mesenchyme. ChiP-Seq data integrated with RNASeq will identify direct PBX transcriptional targets and elucidate how the PBX1-dependent regulatory network controls beta-cell differentiation.

Aim 2 (Years 2/3). To recapitulate the mesenchyme *niche* in a dish to improve the differentiation and maturity of stem cells-derived human beta-cells.

Final year. To characterize the molecular and functional properties of stem-cell derived beta-cells using newly identified mesenchymal factors.

The PhD student will acquire cutting-edge techniques in iPSCs culturing, confocal microscopy, transcriptome and chromatin analyses established in the Spagnoli lab. and bioinformatics skills in the Vigilante lab.

One representative publication from each co-supervisor:

Escot S, Willnow D, Naumann H, Di Francescantonio S, Spagnoli FM. Robo signalling controls pancreatic progenitor identity by regulating Tead transcription factors. Nature Communications. 2018 9(1):5082. doi: 10.1038/s41467-018-07474-6.

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.

33.1 Live imaging and genetic dissection of basement membrane development and repair.

Co Supervisor 1A: Brian Stramer
Research School/ Division or CAG: Folsm/Basic and Biomedical
Email: brian.m.stramer@kcl.ac.uk
Website:
https://www.kcl.ac.uk/lsm/research/divisions/randall/research/sections/motility/stramer/index

Co Supervisor 1B: Claudia Linker
Research School/ Division or CAG: Folsm/Basic and Biomedical
Email: claudia.linker@kcl.ac.uk
Website:
https://www.kcl.ac.uk/lsm/research/divisions/randall/research/sections/motility/linker/index

Project Description:

The basement membrane (BM) is crucial for maintaining nearly all epithelial tissues. It is composed of a range of enzymatically cross-linked matrix components, all produced by a combination of cell-types. Secretion of these components during development leads to their binding to the basal surface of epithelial cells where they are hypothesised to organise largely through self-assembly mechanisms. However, to date the BM has mostly been studied using *in vitro* cell culture or biochemical systems of limited physiological relevance. In this project we will exploit our ability to live image deposition and remodelling of BM components during embryogenesis and repair in Drosophila to dissect the mechanisms involved in its formation in vivo. Furthermore, we will use this system to model human BM disease by generating targeted mutations in BM components. The PhD candidate will initially develop new genetic tools to live image basement membrane components, including targeted mutations that mimic human congenital diseases. They will subsequently examine the role and regulation of these components during a number of developmental processes and injury responses in vivo.

Skills: advanced imaging techniques, Drosophila genetics, molecular biology, quantitative image analysis.

One representative publication from each co-supervisor:

Matsubayashi Y, et al., (2017) A Moving Source of Matrix Components is Essential for De Novo Basement Membrane Assembly. Curr. Biol. 20;27(22):3526-3534.

Richardson et al., (2016) Leader Cells Define Directionality of Trunk, but Not Cranial, Neural Crest Cell Migration. Cell Reports 31;15(9):2076-2088

34.1 Ears in a dish: modelling human ear formation and disease in organoids.

Co Supervisor 1A: Andrea Streit
Research School/ Division or CAG: FoDOCS; Dental CAG
Email: andrea.streit@kcl.ac.uk
Website:
https://www.kcl.ac.uk/dentistry/research/divisions/craniofac/researchgroups/streitlab/streitlab

Co Supervisor 1B: Dr Andrea Serio
Research School/ Division or CAG: Craniofacial Development & Stem Cell Biology
Email: andrea.serio@kcl.ac.uk
Website: https://kclpure.kcl.ac.uk/portal/andrea.serio.html

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 and using multiplex automated 3D imaging to analyse normal and disease-related organoids.

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). S/he will then characterise normal ear morphogenesis and cell type specification using multiplex 3D imaging (year 2). Finally, the student will generate a model for Branchio-oto-renal syndrome using Crispr/Cas9 to replicate mutations newly identified in BOR-patients and analyse the phenotype in ear organoids.

The project will advance our knowledge of human ear formation, provide a new model to analyse disease phenotypes and define the molecular, cellular and morphogenetic consequences of human mutations associated with BOR syndrome. 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:

A systems level approach reveals new gene regulatory modules in the developing ear
Chen, J., Tambalo, M., Barembaum, M., Ranganathan, R., Simoes-Costa, M., Bronner, M. E. & Streit, A., 15 Apr 2017, In : Development (Cambridge): for advances in developmental biology and stem cells. 144, 8, p. 1531-1543

A high-content platform to characterise human induced pluripotent stem cell lines
Leha, A., Moens, N., Meleckyte, R., Culley, O. J., Gervasio, M. K., Kerz, M., Reimer, A., Cain, S. A., Streeter, I., Folarin, A., Stegle, O., Kielty, C. M., Durbin, R., Watt, F. M., Danovi, D. & HipSci Consortium, Mar 2016, In : Methods. 96, p. 85-96

35.1 Predictive Immune Atlas of Cancer Resistance to Radiotherapy.

Co Supervisor 1A: Prof Mahvash Tavassoli
Research School/ Division or CAG: Centre for Host Microbiome-Interaction
Email: mahvash.tavassoli@kcl.ac.uk
Website: https://kclpure.kcl.ac.uk/portal/mahvash.tavassoli.html

Co Supervisor 1B: Dr Shahram Kordasti
Research School/ Division or CAG: School of Cancer and Pharmaceutical Sciences/ Comprehensive Cancer Centre
Email: shahram.kordasti@kcl.ac.uk
Website: https://kclpure.kcl.ac.uk/portal/shahram.kordasti.html

Project Description:

Radiation therapy is the pillar of standard therapy for many types of solid cancers however, resistance to the ionizing radiation is a current problem in the treatment and clinical management of various cancers. The biological and molecular mechanisms responsible for resistance of the tumours to radiotherapy remain unknown. Recently the immune system in tumour microenvironment has been found to influence patient response to RT and disease outcome. Further investigation is necessary to take advantage of these mechanisms in order to develop more tailored therapeutic strategies.

The primary objective of this study is to perform deep phenotyping of immune cells in HNC tumour microenvironment to identify an immune signature to predict RT response

Flowcytometry (conventional and mass cytometry, CyTOF), following Tumour biopsy dissociation (using BD Tumour Dissociation Reagent), will be used as the primary methods to identify specific immune signatures for the prediction of disease progression and RT response. Unbiased data analysis will be performed by the supervisor's already established pipeline to identify immunophenotypic heterogeneity within tumour microenvironment. This pipeline includes dimension reduction by T-distributed Stochastic Neighbour Embedding (t-SNE) followed by a clustering algorithm (ie. SPADE or FlowSOM) based on t-SNE scores. In house developed pipeline (CytoClustR) will then be used to further characterise the identified cell clusters and compare the frequency and expression intensities among samples. This pipeline can be used for both multidimensional conventional cytometry (ie BD Symphony) as well as mass cytometry (CyTOF).

Once dominant cell clusters are identified within the tumour, we will calculate the non-redundancy scores (NRS) for markers which define each cluster and the markers with highest NRS will be used for the sorting of identified cell clusters. The sorted cells will be used in downstream experiments including functional assays.

This interdisciplinary project will provide in-depth training in cancer biology/ immunology, and in a range of cellular, molecular, imaging, biochemical and bioinformatics methods.

One representative publication from each co-supervisor:

Ng T, Pezzella F, Guerrero-Urbano T and Tavassoli M. Br J Cancer. 2017 Apr 11;116(8):1057-1064. doi: 10.1038/bjc.2017.66. Epub 2017 Mar 21.

Deep phenotyping of Tregs identifies an immune signature for idiopathic aplastic anemia and predicts response to treatment. Kordasti S, Costantini B, Seidl T, Perez Abellan P, Martinez Llordella M, McLornan D, Diggins KE, Kulasekararaj A, Benfatto C, Feng X, Smith A, Mian SA, Melchiotti R, de Rinaldis E, Ellis R, Petrov N, Povoleri GA, Chung SS, Thomas NS, Farzaneh F, Irish JM, Heck S, Young NS, Marsh JC and Mufti GJ.
Blood. 2016 Sep 1;128(9):1193-205. doi: 10.1182/blood-2016-03-703702. Epub 2016 Jun 8.

36.1 Understanding compromised wound healing in the ear drum.

Co Supervisor 1A: Prof Abigail Tucker
Research School/ Division or CAG: Craniofacial and Regenerative Biology
Email: abigail.tucker@kcl.ac.uk
Website: https://www.kcl.ac.uk/people/abigail-tucker

Co Supervisor 1B: Dan Jiang
Research School/ Division or CAG: Otolaryngology, Head & Neck Surgery, Guys and St Thomas's Hospital. Honorary Prof: Craniofacial Development & Stem Cell Biology, KCL
Email: dan.jiang@kcl.ac.uk
Website: http://www.guysandstthomas.nhs.uk/our-services/consultant-profiles/audiology/dan-jiang.aspx

Project Description:

This project aims to answer questions relating to how the ear drum heals during normal repair, and how repair mechanisms are influenced in the presence of otitis media (glue ear).

Ear drum perforations are common but generally heal spontaneously, highlighting the regenerative capacity of this tissue. Chronic ear drum perforations do not heal and can cause otalgia, tinnitus and hearing loss.

Factors that have been proposed to influence healing include perforation size, location and the presence of infection/inflammation.

This proposal investigates wound healing in transgenic mouse models, allowing cells to be tracked during the wound healing process (Aim 1). Importantly the project involves mouse models of otitis media, to assess how middle ear disease impacts on healing (Aim 2). Finally we aim to enhance repair by manipulating the pathways that act during the natural healing of the ear drum in order to improve healing in cases of otitis media (Aim 3).

Aim 1: To understand how size and position of holes influence healing

Aim2: To understand how Otitis media and macrophages influence repair

Aim 3: To stimulate repair process in vivo in OM model

Overall this proposal presents a novel way to understand healing of the ear drum and the cells involved in the process, and provides a starting point for more translational activities to prevent chronic perforations developing in patients.

Skills training: The student will be trained in molecular biology techniques, stem cell biology, 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, 1453-1456.

Eze N, **Jiang D**, O'Connor AF. (2014) The atretic plate – a conduit for drill vibration to the inner ear. **Acta Otolaryngol.** 134(1):14-8.

37.1 Improving muscle function in a muscle wasting disorder.

Co Supervisor 1A: Prof Peter Steven Zammit
Research School/ Division or CAG: Randall Centre for Cell and Molecular Biophysics
Email: peter.zammit@kcl.ac.uk
Website: https://www.kcl.ac.uk/people/peter-zammit

Co Supervisor 1B: Elisabeth Ehler
Research School/ Division or CAG: School of Cardiovascular Medicine and Sciences
Email: elisabeth.ehler@kcl.ac.uk
Website:
https://www.kcl.ac.uk/lsm/research/divisions/randall/research/sections/signalling/ehler/ehlerelisabeth

Project Description:

Muscular dystrophies are characterised by muscle weakness and wasting. Facioscapulohumeral muscular dystrophy (FSHD) is caused by ectopic expression of a transcription factor called DUX4. FSHD muscle cells are sensitive to 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 (RNA-Seq) data from muscle and immune cells has implicated several mediators of oxidative stress and mitochondrial generation, along with pathways controlling inflammation. For example, we have identified the PGC1alpha/ERRalpha axis as suppressed in FSHD and have found compounds/nutritional supplements that target this pathway, improving 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: Examine expression dynamics and manipulate pathways that we have identified that are 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 and determine if protect against oxidative stress and inflammation in FSHD and their effects on 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.

38.1 The molecular basis of intellectual disability.

Co Supervisor 1A: Vlad Seitan
Research School/ Division or CAG: BMBS
Email: vlad.seitan@kcl.ac.uk
Website: https://www.kcl.ac.uk/people/vlad-seitan
https://www.kcl.ac.uk/lsm/research/divisions/gmm/departments/mmg/researchgroups/seitanlab/index

Co Supervisor 1B: Richard Wingate
Research School/ Division or CAG: MRC Centre for Neurodevelopmental Disorders
Email: richard.wingate@kcl.ac.uk
Website: https://www.devneuro.org/cndd/

Project Description:

Mutations in the *ZBTB11* gene cause a rare syndrome characterised by intellectual disability, cerebellar atrophy and neuromuscular defects. The molecular and cellular functions of Zbtb11 are still poorly understood, precluding our understanding of the mechanisms underpinning this condition. Our lab has developed state-of-the-art *in vitro* and *in vivo* experimental models to study the functions of Zbtb11, and we use inter-disciplinary approaches including functional genomics, mouse genetics and biochemical techniques, to determine the precise cellular and developmental pathways controlled by this factor.

This project will focus on understanding the roles of Zbtb11 in the development of the cerebellum. *ZBTB11* is particularly highly expressed in this part of the brain, and the cerebellar abnormalities and neuromotor defects displayed by patients with *ZBTB11* mutations, indicate an important function for this factor in the development and activity of the cerebellum. The student will have access to a newly established mouse line in which Zbtb11 is specifically deleted in the developing cerebellum, and will be able to leverage expertise in functional genomics/transcriptional regulation (Seitan lab) and cerebellum development and function (Wingate lab). The aims will be to determine which stage in cerebellum development is dependent on Zbtb11 (year 1), subsequently use whole transcriptome analyses to identify the genes and pathways controlled by Zbtb11 in the developing cerebellum (year 2), and to validate the findings through functional assays (years 2-3). The project will offer training opportunities in mouse genetics, flow cytometry, microscopy, and functional genomics technologies such as RNA-seq and ChIP-seq, including computational techniques.

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

Wilson, Brooke C., Lena Boehme, Ambra Annibali, Alan Hodgkinson, Thomas S. Carroll, Rebecca J. Oakey, Vlad C. Seitan (2019) ‘Intellectual Disability-Associated Factor Zbtb11 Cooperates with NRF-2/GABP to Control Mitochondrial Function’. BioRxiv, 2019.12.13.875708.
https://doi.org/10.1101/2019.12.13.875708

Hanzel, M., Rook, V., Wingate, R.J.T., 2019. Mitotic granule cell precursors undergo highly dynamic morphological transitions throughout the external germinal layer of the chick cerebellum. Scientific reports 9, 15218.
https://www.ncbi.nlm.nih.gov/pubmed/31645601