KCL PhD Programme in Biomedicine & Bioinformatics - Project Catalogue 2022

This document contains exemplar projects for the 2022 PhD programme in Biomedical Science & Bioinformatics to choose from for MRes rotations and final PhD project.
Please be aware that projects are subject to change and may not all be available for a final PhD.

Contents

Project 1 - The crosstalk Between The Primary Tumour and The Tumour Draining Lymph Node in Post-Neoadjuvant Chemotherapy (NACT) Treated Triple Negative Breast Cancers

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Abstract

In this project, we will study the two-way communication between the primary tumour and the tumour draining lymph nodes (LNs) in post-NACT treated triple negative breast cancers (TNBC), histologically defined by the absence of estrogen receptor (ER), progesterone receptor (PR) and c-erbB2 (HER2) receptors. TNBC's occur more commonly in younger women and those of African-American ethnicity, and are characterised by their clinically aggressive nature. In comparison to other breast cancer subtypes, the interaction between cancer cells and the tumour-immune microenvironment in TNBC has been shown to alter prognosis. However, despite recent therapeutic advances, TNBC remains hard-to-treat. NACT followed by surgery is currently the mainstay treatment for early stage TNBC. However, the response rates to conventional NACT are modest and there is a lack of response biomarkers. Targeted therapies are emerging for subgroups of TNBC in the adjuvant and metastatic disease setting, and the combination of chemotherapy with anti-PDL1 and anti-PD1 immunotherapy has shown encouraging results in early-stage TNBC.

The assessment of Tumour-Infiltrating Lymphocytes (TILs) at the primary tumour is superior to classical Tumour Node Metastasis (TNM) staging in TNBC in predicting outcome, response to chemotherapy, and immunotherapy. In this context, the quantification of TILs in stromal tissue within tumour before and after chemotherapy (in the residual tumour) revealed that high TILs density (>30%) predicted response to therapy and survival benefit. However, only one-third of TNBCs have high TIL density and a low level of TILs does not equate to disease progression. We uncovered that increased germinal center (GC) reactions across cancer-free axillary LNs of TNBC patients is significantly associated with a good prognosis. This is the case particularly when TIL density is low or absent in the primary tumour. Notably, TNBCs with high TILs invariantly displayed GCs in their cancer-free axillary LNs. The finding that GC reactions in LNs significantly associates with superior outcomes even when TILs are absent represents the first known prognostic immune biomarker readout in cancer-free LNs. Overall, this knowledge prompts the need to look at the primary tumour and at the tumour draining LNS, for the building of a multidimensional immune-atlas post-NACT TNBC.

So far, we have established several methodological approaches to study the crosstalk between the tumour and LNs, including mouse models of breast cancer, microfluid devices to test chemotaxis of ex-vivo slices of tumours and lymph nodes, as well as ex vivo normothermic perfusion. In this project, we will utilise tumours and LNs from chemo- and immuno-therapeutically treated animals and TNBC patients. We are interested to explore how these therapeutics influence the LNs crosstalk to the tumour. Through the KHP Biobank in the Cancer Centre, we have access to hundreds of fresh frozen and FFPE tumours and lymph nodes from neoadjuvantly treated TNBC patients with long-term follow-up data.

By understanding the intimate crosstalk between the tumour and the LN, the signals required for the occurrence of TILs at the primary tumour, we will obtain new insights into predicting the outcome of therapies, identify novel targets for immune potentiation, and unleash truly novel therapeutic strategies for a high-risk breast cancer patient group.

Project 2 - Uncovering molecular mechanisms that link maternal diet to lifelong adipose storage and metabolic disease

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Abstract

In England obesity is rising in the population and by 2050 is forecast to affect between 50-60% of adults and 25% of children. As body mass index (BMI) rises, so do the risks for coronary heart disease, stroke, and Type 2 Diabetes. Whilst excess weight is risky for all, some people remain relatively healthy despite being obese, whereas others develop metabolic diseases. This is partly due adipose depot location, since people who store fat in their abdomen are more likely to develop complications of obesity than those who store it under the skin. It is not known why people vary in this respect, but at least part of the mechanism is thought to be due differences in exposure to a compromised intrauterine environment. In the UK currently ~50% of mothers are overweight or obese when entering pregnancy. Both epidemiological and animal model studies have demonstrated that maternal obesity is a major risk factor for offspring metabolic disease. However, molecular mechanisms linking early life exposure to adult metabolic dysfunction, including compromise adipose expansion, are poorly understood.

In humans and in animal models, a pool of cells that can become fat cells (adipose precursor cells or APCs) are generated in early life. The size of this pool in each location remains fairly constant throughout life, but some people have a larger pool of APCs in some depots than others, and this determines how and where their fat expands. If the APC pool is limited, adipose depots cannot produce new cells, and instead excessive fatty acids must be stored in larger fat cells (hypertrophy) or ectopically in other peripheral organs such as liver and muscle. Hypertrophic adipose tissue and ectopic fat storage is proinflammatory and causes increased peripheral insulin resistance – leading to increased risk of heart disease and Type II Diabetes.

We isolated APCs from adipose depots of young mice exposed to maternal obesity during pregnancy, as well as APC from offspring of lean mothers. Preliminary analysis of RNA sequencing data indicated profound changes in multiple developmental signalling pathways in response to maternal diet, and some of these responses were sex-specific. The student will use transcriptomics tools to evaluate i) gene regulatory networks associated with the APC response to maternal diet; ii) the role of alternative splicing in dietary response of these stem cell populations. Emergent hypotheses will be tested in a comprehensive data/sample set generated by the host lab where embryonic and postnatal adipose tissue was collected from both lean and obese pregnancies. This material will also be used to determine if epigenetic pathways drive differences between dietary groups. Ultimately, candidate pathways will be explored in human population data using Genomics England resources.

By investigating genetic and epigenetic pathways that control how cells decide to become APCs during development we hope to understand individualised metabolic risk as a result of obesity – a topic with currently considerable confusion in the public health arena. In addition, we hope that in the future we might be able to develop drugs that promote healthy fat storage.

Project 3 - Investigating the crosstalk between the intestinal epithelium and immune cells in health and disease

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Abstract

The mammalian gastrointestinal mucosa is a unique environment colonized by a highly complex mixture of microorganisms that establish a mutualistic relationship with the host. The defense system of the intestinal mucosa comprises an epithelial layer and a plethora of immune cells that restrict commensals within the intestine while preserving their number and diversity. To maintain gut homeostasis the intestinal epithelium secretes a variety of mediators that control immune cell functions as well as the composition and stratification of commensal bacteria. Conversely, intestinal epithelial cells (IECs) respond to cytokines and other mediators secreted by immune cells which in turn control IEC function and regeneration. Dysfunction of the intestinal barrier and epithelial-immune crosstalk are associated with a variety of local and systemic pathologies including Inflammatory Bowel Disease (IBD), which affects over 500,000 people in the UK (1 in 133). IBD comprises a mixture of intestinal inflammatory conditions, primarily represented by Crohn's disease and ulcerative colitis. These are lifelong conditions without a medical cure, costing the NHS ~£1 billion/annum. Patients are commonly treated with immunosuppressive drugs, but up to 30% of patients remain unresponsive to treatment and will undergo surgery normally involving the removal of the affected bowel. The factors triggering the development of IBD are incompletely understood, but the balance between health and disease is most likely dictated by the crosstalk between immune and epithelial cells. Hence, understanding the mechanisms controlling intestinal epithelial integrity, function and regeneration is critical to identify new pathways for the treatment and prevention of IBD. In this project we aim to explore the immune- mechanisms controlling IEC fate and function and to provide understanding of the pathways regulating the immune-epithelial crosstalk and how they shape intestinal homeostasis and the development of IBD. These studies will identify novel mechanistic links between epithelial and immune pathways which could have wide-ranging consequences for diagnosis and therapeutics for intestinal diseases.

Project 4 - Pathogenic mast cells in chronic inflammatory diseases

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Abstract
Mast cells (MCs) abound in all barrier tissues and play an important role in pathogenesis of many chronic inflammatory diseases including urticaria, atopic dermatitis, polyposis and food allergy. Mediators produced by MCs are thought to drive inflammation, tissue remodelling and pathological growth (polyps). It is known that MC phenotype is determined by local environment and thus extensive heterogeneity of MC is predicted in human tissues but as yet poorly studied and understood. We studied immune cells obtained from nasal polyps and found increased percentages of MC expressing IL-17RB (receptor for IL-25), identifying a potentially novel MC subpopulation. Our observation of markedly increased IL-17RB+ MCs in nasal polyps could suggest that "pathogenic" MCs are important for regulation of severity of chronic inflammation. Our aim is to use an unbiased approach to confirm features of IL-17RB+ MCs and to comprehensively evaluate MC heterogeneity at the single cell transcriptomic and epigenetic levels. As extensive heterogeneity of single cell RNA sequencing data is known to be a potential problem, to further define MC subpopulations at the genomic level, chromatic regulatory landscape will be analysed in parallel to transcriptomic analysis at the single cell level. It is well known that chromatin landscape usually presents a more stable picture of cell status in comparison to transcriptomics, helping to define unique cell differentiation states (subpopulations). Combination of single cell RNA sequencing data with single cell epigenetic patterns will allow us for the first time to fully characterise human MCs. Identified MC subpopulations (signatures) will be further researched using already obtained transcriptional data from chronic urticaria patients, healthy controls and other available datasets of patients with chronic inflammatory diseases. To characterise functions of IL-17RB+ MCs a model of human MCs overexpressing IL-17RB has been established and will be used to study the role of IL-25/IL17RB in MC activation. Although there are many therapies targeting immune cells, there are no current treatments that specifically target MCs or MC specific pathways. This project will generate novel data regarding human subpopulations of MCs using cutting-edge cellular and molecular techniques. Our data will generate a blueprint for MCs expressing IL-17RB and demonstrate whether IL-25 pathway is likely to be important in determination of MC phenotype and function. We anticipate that our data will provide a strong basis for a larger follow-up research programme aimed at exploring MC heterogeneity and MC targeted therapies for chronic inflammatory diseases

Project 5 - Identifying mechanisms of resistance to targeted therapies in Acute Myeloid Leukaemia (AML)

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Abstract
Chemotherapy remains the mainstay of treatment for AML but is associated with severe side effects and poor long-term survival. Novel targeted therapies for AML are in development and some have recently been approved, providing potential to substantially improve outcomes. However, they are invariably associated with resistance, the mechanisms of which remain poorly understood, representing a major barrier for achievement of long-term cure. Resistance mechanisms are likely to vary between agents and between molecularly-defined disease subgroups. The overall aim of this project is to perform deep molecular characterisation of matched samples taken at diagnosis and relapse after targeted therapy to identify novel markers predicting resistance to therapy, and to validate these experimentally, both for development as biomarkers to inform treatment selection and as drug targets which could be themselves targeted to overcome resistance.

1) Identifying mechanisms of resistance to BCL2 and Menin inhibition in KMT2A-rearranged AML
KMT2A-rearranged AML is common in children and younger adults and is associated with poor survival. Agents targeting BCL2 and Menin both show promising efficacy in early-phase studies but produce only transient responses. Preliminary data generated from patients in the NCRI-AML19 and CRUK-MyeChild studies using whole transcriptome sequencing performed in the BRC reveal upregulation of genes involved in drug metabolism, including members of the Flavin-Containing Monooxygenase (FMO) family that catalyse the oxidation of heteroatom compounds, and as such could plausibly metabolise the BCL2 inhibitor, venetoclax (figure 1). To validate these FMO proteins as attractive therapeutic targets, the student will initially deepen this analysis with a larger number of patients, and apply orthogonal techniques including sequential whole genome sequencing (to exclude confounding by acquisition of new mutations) and chromatin profiling by ATAC-seq and Cut&Run of histone marks. A separate cohort of patients who have paired samples before and after treatment with the Menin inhibitor JNJ-75276617 (currently in a BRC phase 1 trial) will be evaluated using the same methodology. Candidate drivers of resistance will be manipulated in genetically-relevant cell lines, including overexpression and CRISPRCas9 knockout to test whether this can modulate sensitivity to the relevant drug.

Whole transcriptome analysis of patients with KMT2A rearranged leukaemia relapsing after BCL2 inhibition identifies increased expression of key genes involved in drug metabolism,

2) Identifying mechanisms of resistance to targeted inhibitors of FLT3 FLT3 mutations occur in ~30% of AML. Targeted FLT3 inhibitors produce clinical remission in ~40% of patients; however, almost all patients progress and die. In a minority of cases resistance is associated with acquisition of additional mutations in FLT3 or in RAS pathway genes but in most cases is unexplained suggesting an epigenetic mechanism of resistance. The student will apply the methodologies described in objective 1 to samples taken at initiation and subsequent failure of FLT3 inhibitor therapy. As an orthogonal approach, the student will apply CRISPR-dropout screening to FLT3-inhibitor resistant cell lines (including the MOLM-13-RES cell line that is resistant to the FLT3-inhibitor MLN518). Candidate targets will then be manipulated experimentally to determine if sensitivity can be restored.

3) Using single cell approaches for early detection of emerging resistance Having established markers of resistance the bulk level in patients with frank haematological relapse, the student will apply single cell techniques to detect these in small cell populations at pre-clinical relapse where patients have measurable residual disease (MRD) but remain in clinical remission. Single cell RNAseq (scRNAseq) allows the detection of residual leukaemia cells and allows their transcriptional profiles to be interrogated. By applying this to sequential samples, the student will investigate whether particular expression clusters correlate with drug resistant phenotypes (fig 2), allowing identification of novel biomarkers to guide molecularly targeted intervention in the MRD+ setting to prevent relapse.

Figure 2. Example of scRNAseq from samples pre- and post-treatment from a patient with NPM1-mutant AML. NPM1-mutant cells are detected and marked blue, whereas wild-type cells are in yellow.

Project 7 - Investigating human B cells and their expressed antibodies in patients with solid tumours to evaluate antigen reactivity

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Abstract
Despite the success of cancer immunotherapy, a proportion of patients develop resistant disease or significant life-threatening toxic effects. Biomarkers for patient stratification alongside new therapy options including tumour-specific antibodies could transform patient care and outcomes. Human B cells and their expressed antibodies are crucial in conferring immune protection against pathogens, however, in the last decade, there has been a growing appreciation of their roles in cancer, especially in immunogenic malignancies such as melanoma. Identifying pathogen-specific B cells following infection is possible due to enhanced humoral immunity against well-described molecules on the pathogen surface, while screening for cancer-reactive B cells and their expressed antibodies remains challenging since the antigen reactivities of B cells in cancer patients remain insufficiently elucidated. Furthermore, the involvement of humoral immunity in clinical outcomes and response to therapy remains unclear. We have shown that innate signals, alongside specific cancer antigen stimulation, in individuals with and without detectable circulating tumour antigen-specific antibodies, support prolonged survival of differentiated, memory and often antigen-educated human B cells. We found specific B cell populations most likely responsible for producing specific antigen-reactive antibodies. The intracellular mechanisms regulating and promoting B cell differentiation and antibody secretion remain unexplored in tumour-reactive B cells. Importantly, these processes have not been studied in the context of cancer patient immunity. Dissecting these will be key to better understand and expand antibody secreting cells for the development of novel anti-cancer therapies.

The aim of this project will be to identify specific B populations that secrete cancer antigen-reactive antibodies and to gain insights into the processes that regulate antibody production in these B cell subsets. These B cell subsets may be important to help boost or expand an anti-tumour immune response in patients as part of therapy or could be a source of anti-tumour antibodies which could be cloned and studied for their therapeutic efficacy. Based on our established ex vivo innate signal and antigenic stimulation pipeline, we will explore the B cell subsets derived from cancer patients and healthy subjects using an in-house developed flow cytometry panel (CyTOf) and long-read antibody sequence analyses. We will expand B cells ex vivo and evaluate patient-derived B cell culture supernatants for the presence of antibodies reactive to cancer antigens. Specifically mapping protein pathways known to be responsible for antibody secretion against cell subsets will allow us to evaluate how antibody production is regulated in human B cells from cancer patients. Moreover, these insights will be used to identify more precisely antibody-secreting cell (ASC) populations which express tumour antigen-reactive antibodies.

We expect that the knowledge acquired will advance our understanding of the processes that regulate antibody production and secretion by B cells which react to specific cancer antigens and will help identify antigen-reactive B cells in patients with cancer. This project harbours potential to expand the potential treatment options for patients with melanoma beyond existing therapies.

References:

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Egbuniwe IU et al., J Invest Dermatol. 2022;142(3 Pt A):726-731
Karagiannis P et al., Clin Exp Immunol. 2021;207(1):84-94

Project 9 - Understanding the causes of hyperglycaemia in pregnancy; towards precision medicine in gestational diabetes

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Abstract
Gestational diabetes (GDM) defined by new onset hyperglycaemia in pregnancy, affects ~14% of pregnancies worldwide and prevalence is increasing. Long-term morbidities include progression to prediabetes/type 2 diabetes in ~50% of affected mothers within 10 years. The description of GDM as 'new hyperglycaemia in pregnancy' and a yes/no diagnosis at 24-28 weeks' gestation suggests homogeneity, reinforcing the dogma of a single pathological entity. This 'one size fits all' approach is discordant with the observed clinical diversity, including variation in time of onset, hyperglycaemic patterns and treatment response. Better understanding of the biological pathways that distinguish GDM subgroups will facilitate a personalised approach to management.

This project, using data collected as part of the MRC-funded UNiCoRN Study (Understanding the causes of Hyperglycaemia in Pregnancy; MR/W003740/1) will explore pathophysiologically distinct subgroups leading to hyperglycaemia in a cohort comprising women of White and South Asian descent. Disease subtypes will be elucidated through integration of routine maternal and clinical data with targeted hormonal profiles captured at oral glucose tolerance tests in early and mid-pregnancy, and the gestational glucose profile measured using continuous glucose monitoring. This study will lay the foundation for personalised and physiologically targeted therapy for GDM and later prevention of type 2 diabetes, leading to improved outcomes for mother and child.

Project Objectives will include:

1. Identification of factors (clinical/biochemical) that distinguish between subtypes of GDM at 24-28 weeks' gestation to facilitate targeted management
2. Identification of factors (clinical/biochemical) in early pregnancy associated with GDM subtypes to facilitate earlier targeted intervention
3. Exploration of distributions of GDM subtypes across White and South Asian groups
4. Exploration of behavioural influences (e.g. diet/physical activity) amongst women from White and South Asian groups on the development and phenotype of GDM

Project 10 - Nanoneedle-mediated transfection for CAR-Treg manufacturing

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Abstract

RATIONALE:
Cell therapies using chimeric antigen receptors Tregs (CAR-Tregs), genetically engineered for antigen-specific targeting, are a promising approach to modulate immune response (e.g. transplant, autoimmune diseases), aimed at reducing reliance on immunosuppressants. Despite the therapeutic success of CAR-Tregs therapy, state-of-the-art methods for CAR-Treg generation rely on viral transduction limiting the size of the insert, requiring labour and cost-intensive procedures and showing undesirable batch-to-batch variability, reducing accessibility to this therapy.

Nanoneedles can provide a way to engineer CAR-Tregs at low cost, with high efficiency, high throughout, low variability, and minimal toxicity. A nanoneedle patch is a large array of vertical nanoscale spikes emerging from a surface. Nanoneedle patches used as cell culture substrates are capable of efficient genetic engineering of primary cells using DNA, RNA and gene editing constructs (CRISPR, transposons).

OBJECTIVES:
This project develops our nanoneedle platform to generate CAR-Tregs, with the objective to obtain superior transduction efficiency than the established approach, higher reproducibility and a broader range of gene editing approaches (e.g. CRISPR, prime editing), while retaining Treg cell functionality.

Year 1: Establish the nanoneedle platform for Treg transfection
Year 2: Optimise efficiency and reproducibility for CAR-Treg generation with nanoneedles.
Year 3: Evaluate performance of nanoinjected CAR-Tregs and compare with transduced CAR-Tregs.
SKILLS:

The student will develop unique skills learn in the nanoneedle transfection of Tregs. They will also learn primary T-cell culture, genetic engineering techniques including nanoneedle delivery, bioengineering techniques for nanoneedle preparation, cell and molecular biology assays to assess Treg transduction, viability and function including qPCR, WB, immunofluorescence, flow cytometry and T-cell activation assays. Trainings for these techniques established in the respective labs.

Project 11 - Is quiescence index of prognostic value to glioblastoma patient outcomes?

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Abstract
Glioblastoma multiform (GBM) is the most common and aggressive of malignant primary brain tumours, with average patient survival of ~12-15 months post-diagnosis, given universal recurrence after standard treatment (surgery followed by concomitant radiotherapy and chemotherapy). GBM patients respond differently to current therapies, some not at all. These treatments are highly toxic, so we would want to foresee who might benefit, moving towards personalised medicine. This project investigates the hypothesis that magnitude of GBM stem cell (GSC) quiescence correlates with therapy response, relapse and survival times.

GBM is driven by neural stem cell (NSC)-like cells so a cure requires eradicating them. This is particularly difficult because, unlike NSCs, GSCs proliferate indefinitely and are infiltrative; furthermore, a large fraction of GSCs are quiescent, which confers therapy-resistance. Quiescence (also known as G0) consists in reversible cell-cycle arrest accompanied by low biosynthesis, which protects cells from replicative exhaustion, proliferation-induced mutations, and environmental insults.

Quiescent cancer stem cells evade cytostatic and even immune therapies. Therapy-resistant quiescent GSCs (qGSCs) can reactivate and reinitiate GBM, prompting the untested hypothesis that qGSC magnitude may correlate with patient prognosis. We have recently refined a marker cocktail and protocols to identify and quantify active and qGSCs in human GBM tissue. We will exploit this to determine the quiescence index (QI: fraction of GSCs that are qGSCs) in hundreds of GBM for which we will have associated clinical data. These will come from the neurosurgery service at KCH (led by Supervisor 2) or Brain UK.

To determine QI, the student will apply established histological protocols to freshly-collected or banked tissue, and in parallel will develop a tissue dissociation, staining and fluorescent-activated cell sorting method in collaboration with the BRC Flow Cytometry platform, which will increase throughput. Power calculation assuming linear relationships between GBM patient outcome measures and QI, plus normal distributions, indicates that 85 samples will detect correlations up to 0.3 with 80 % power and 5 % significance level. The necessary sample size should be attainable for wild-type isocitrate dehydrogenase (IDH) 1/2 patients, with >150 expected over 3 years from KCH alone. IDH mutations are found in in ~10 % GBM and double median survival so determination of IDH genetic status is part of current standard of care. For each tumour within either IDH category, QI will be matched to progression-free and overall survival times.

We will employ the Cox proportional hazard model used in survival analysis to link clinical covariates (such as QI, age, tumour volume, treatment regime) with those outcome measures; and Bayesian inference to fit

model parameters with and without the contribution of QI to determine if the latter is of prognostic value. With this analysis we will also be able to quantify the differential roles of other clinical covariates in shaping outcome measures and to find associations between QI and other clinical features. For example, is there association between QI and IDH status or are these orthogonal predictors of patient outcome? Modelling and statistical analyses will be supported by the BRC Biostatistics and Data Management Platform.

Project 12 - Establishing the role of succinate dehydrogenase (SDH) gene mutations in pituitary tumourigenesis

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Abstract
Succinate dehydrogenase consists of four subunits and is a key respiratory enzyme linking the Krebs cycle and the electron transport chain. Patients with germline mutations in one of the five succinate dehydrogenase genes (SDHx) or the SDH subunit assembly factor SDHAF2, typically present with paragangliomas and phaeochromocytomas, as well as additional tumours, including pituitary adenomas. Pituitary tumours associated with either germline or somatic SDHx mutations, in particular SDHB, can display clinically aggressive behaviour. The molecular pathogenesis, clinical behaviour and treatment outcomes of SDHx-related pituitary tumours remain poorly characterised. There is a clinical need to better understand the pathways involved in SDHx-related pituitary tumours, which could inform use of medical, surgical and radiation strategies. This project aims to determine the mechanisms through which SDHx mutations lead to tumour formation and identify if mutation in pituitary stem cells, marked by the transcription factor SOX2, is sufficient to drive tumourigenesis or if the mutations affect committed and differentiated endocrine cells. Understanding the cell-of-origin of these aggressive tumours is crucial in the development of translational approaches, such as identification of specific epitopes that could be utilised in new biotherapies. No chemical treatments are currently in place for these tumours, hence identified signalling pathways implicated in pathogenesis will enable targeting using small-molecule inhibitors.

The project will utilise an efficient primary cell culture system established in the lab, to culture human fetal pituitary stem cells expressing SOX2 (tissue available through the Human Developmental Biology Resource). Established endocrine cell lines across the different lineages are available as a back-up. SDHx genes will be mutated through CRISP/Cas9, recapitulating known patient mutations. Mutant cell cultures will be validated for impaired succinate dehydrogenase activity and assessed for changes in cell behaviour compared to controls, including migration, invasion, and proliferation, as well changes in their phenotype during differentiation. The effects of the different mutated subunits will be compared, with a focus on SDHB if required, since mutations are more likely to lead to an aggressive/metastatic phenotype. Global gene expression and chromatin accessibility changes will be mapped through multi-omic approaches to establish changes in accessibility and predicted regulons driving tumourigenesis. If the opportunity to carry out bulk or single nuclei multi-ome approaches of SDHx mutant tumours arises (depending on sample availability), these data will be included for analysis. Key dysregulated genes will be validated at the mRNA and protein level in human cells, normal pituitary tissue and in archival pituitary tumour samples with and without SDHx mutations and assessed as biomarkers. The function of upregulated genes will be disrupted in mutant human pituitary cell lines with the aim of rescuing the phenotype. Perturbed signalling pathways implicated in SOX2 cell transformation will be chemically disrupted using appropriate small molecule inhibitors. The

findings will be complemented by characterisation of a pre-clinical conditional Sdhb mutation mouse model, which will be supported through additional resources of the host lab

Project 13 - Regulatory T Cells in Pregnancy Adverse Outcomes (Rutepo)

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Abstract
Pregnancy adverse outcomes (PAO) such as pre-eclampsia (PE) (high blood pressure with proteinuria), gestational diabetes (GDM) (high glucose in pregnancy) or preterm birth (PTB) can be devastating for the parents and the newborn child. They can lead to prolonged hospital stays for both the mother and the child. In addition, they can affect the child's future development and lead to other co-morbidities such as cerebral palsy and neurodevelopmental delay, obesity, and lifelong diabetes for both. Immune cells play an essential role in pregnancy as they prevent the rejection of the fetus. One type of these immune cells is called Regulatory T Cells or 'Tregs'. Tregs are very heterogeneous and are composed of many subpopulations characterized by the expression of different markers1. In a recent systematic review2, we concluded that PE is associated with lower circulating Tregs in pregnancy than a healthy pregnancy2. In addition, pregnancies affected with PTB also seem to have a lower number of Tregs3. Furthermore, pregnancies affected by GDM cannot compensate for the increased immune demands during gestation. This results in a state of low-grade inflammation and insulin resistance. An imbalance between immune cells can be identified in pregnancies with GDM4.

Aims
This project aims to study how Tregs and the different subpopulations, can be affected in PE, PTB and GDM in 4-time points during pregnancy and how these Tregs change six weeks after birth. With the potential of identifying a defective immune Treg subset in the early stages of pregnancy, the presence of this biomarker may be used to prevent the development of PAOs by following a therapeutic intervention, lifestyle or dietary changes and careful weight monitoring.

We have already set up the pilot study, supported by a Fetal Medicine Foundation grant (£230k), at the Fetal Medicine Research Institute in Denmark Hill. A total of 1000 pregnant participants5 attending their routine 36-week scan were recruited. About 10% of these patients in our cohort were affected by gestational diabetes, 3% with pre-eclampsia and 1.6% with preterm birth. We have a mixture of various demographics, at the King's Health Partners NHS Trusts. The analysis of these samples is underway using immune profiling expression panels and genetic studies. There is a difference in the immune cells in the cohorts who developed pregnancy adverse outcomes and more specific in Tregs, Th1, Th2 and Th17. In pregnant individuals recruited at their booking visit, we will assess the presence of Treg subsets at 12,20,28,36 and 6 weeks postnatal and compare healthy pregnancies with PAOs.

Study Design and Methods
We aim to recruit an additional total of 1000 patients and collect blood from them at 5-time points. The immune cells from the blood will be isolated and frozen for analysis. To avoid any experimental bias, each

participant's cells will be analysed when all five samples are collected. We will use a modified immune panel and gene analyses designed and tested during the pilot project to study how the immune cells change during the pregnancy, six weeks postpartum and their association with PAOs.

Project 16 - Common mechanisms of fibrosis? Hypertension, Fibroids and Keloid in African-origin women

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Hypertension and related aortic/myocardial stiffening start at a younger age1, and cause heart failure more often in African-Caribbean- (AfC)/ directly African origin-(Af) people than does atherosclerosis, the major cause in Europeans. Curiously, hypertension/cardiac-vascular stiffening coincides frequently with uterine fibroids and keloid scars, both also excessive in AfC/Af. Moreover, coronary microvascular dysfunction closely associates with hypertension and fibrosis2. Based on these observations, we hypothesize that uterine fibroids and keloid share molecular mechanisms of fibrosis with small vessel, aortic and myocardial fibrosis, underlying their links with hypertension. This PhD project will use translational and multidisciplinary methods to test this hypothesis, contributing to an entirely new angle for the origins of heart failure in under-served populations with high disease burdens.

Clinically, AfC/Af women with imminent fibroid or keloid surgery or existing cardiac magnetic resonance (CMR) data whose fibroid status is known will be invited to the research study. Their cardiovascular health will be assessed using standard (e.g., Blood Pressure, BP) and cutting-edge methods (e.g., aortic pulse wave velocity, CMR). The objectives are to:

i. confirm links between fibroids, keloid and higher BP in the women recruited compared with controls;
ii. examine vascular pathology in fibroid, keloid, and hypertension;
iii. build banks of tissue/cells from these conditions (linked with clinical data) for mechanistic laboratory investigations.

The lab-based work involves working with existing cells/tissues already available, and establishing representative cell cultures from uterine fibroid lesions, pathological vasculature (e.g., aorta, arteries/arterioles from fibroids), and appropriate healthy controls. With the overarching aim of identifying common histological, genetic, transcriptional, and/or proteomic features across the three diseases, there are initially three experimental objectives:

1. Analyse archived paraffin blocks (histology samples) representing healthy versus fibrotic aorta/uterus/skin using histological staining highlighting extracellular matrix (ECM) composition and imaging strategies to reveal ECM organisation.

2. Identify common traits and generate hypotheses about common mechanisms across the diseases using bioinformatic approaches. Specifically, we anticipate significant value in assimilating the currently disparate datasets on the genetic, transcriptional, and proteomic (e.g.,3) characteristics of the individual diseases.

3. Characterise the ECM composition/organisation. Using our "cell-derived matrix" culture system that we have established mimics ECM composition and organisational changes in normal and keloid dermal fibroblasts, this will be extended to analyse the 3 'cell-pair' types (normal vs disease).

By investigating common mechanisms of fibrosis in fibroids, keloid and aortic biopsies and their relationship to hypertension, vascular and myocardial stiffening, this proposal aims to identify new targets for diagnosis, risk assessment and strategies for treatment.

References (and example citations from supervisory team)

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Project 21 - Novel inflammation targeted therapy aimed at glucose metabolism, a multiomics profile

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Abstract

Periodontitis is an host modulated inflammatory disease. Bacteria continuously challenge periodontal tissues which result in direct and indirect host-mediated inflammatory damage. Dysbiosis of the microbiota that starts gingivitis and subsequently leads to periodontitis, is driven by alterations to the local environmental conditions, and the major driver of this altered ecology is the inflammatory response of the host. Treatment and prevention for periodontitis focuses on removal of microorganisms, however, inflammatory processes (IP) are not addressed in current strategies for management of the disease.

Recent research demonstrates that glycaemic control (GC) influences metabolic and cellular processes associated with the development of inflammatory conditions, including periodontitis. Metformin is a first-line drug on the treatment of diabetes, in addition to its effects on glucose metabolism, metformin modulates metabolic and cellular processes associated with the development of inflammatory conditions . As such, metformin is of particular interest in clinical translational research in periodontal treatment since it influences fundamental biological mechanisms that underlie multiple reparative and molecular pathways of inflammation.

Since metformin is ready to use and is known to be safe for clinical use, a pilot double blinded randomized clinical trial (RCT) was performed on non-diabetic participants with chronic periodontitis (n=5 850mg Metformin and n=5 placebo) to demonstrate the capacity of metformin to modulate systemic inflammation post periodontal treatment. Our trial has shown that taking metformin systemically during the acute healing phase post periodontal disease treatment decreases blood glucose and high sensitivity C-Reactive Protein (hsCRP) significantly (Figure 1). Therefore, in this project we aim to understand the impact of glucose modulation on periodontal disease prevention by investigating the composition and metabolics of subgingival plaque, gingival crevicular fluid and clinical parameters in patients that undertake metformin or not during clinical experimental gingivitis model. To complement this study, we will use mouse models to investigate transcriptomic differences of periodontal tissues to understand periodontal stem cell modulation when animals have glucose enhancement.

Overall, the project aims to generate a multiomics profile on the effect of glucose metabolism enhancement aimed at modulation of reparative and molecular pathways of inflammation. Therefore, creating a novel preventative/therapeutic strategy to target periodontal disease progression.

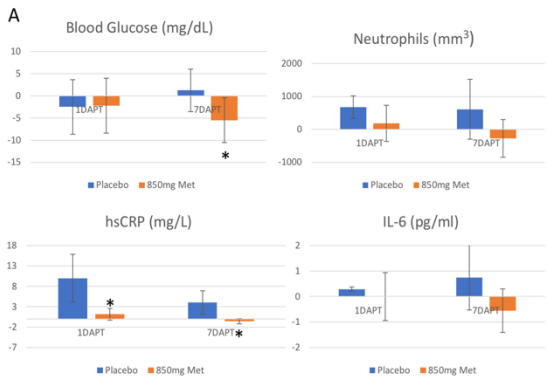

Figure 1. Metformin modulates systemic biological pathways

A)Blood glucose levels significantly decreased 7 days after periodontal treatment on non-diabetic patients taking 850mg metformin (p=0.0297). hsCRP was significantly lower on during the acute phase of treatment on patients taking 850mg Metformin(1DAPT p=0.0412; 7DAPT p=0.0016). Finally, neutrophil count and circulating IL-6 were lower than those on placebo one day after treatment and seven days after treatment

Project 22 - Making fibrocartilage disc cells for repair

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Abstract
The fibrocartilage disc of the temporomandibular joint (TMJ) plays an essential role in cushioning jaw action. The disc, like many fibrocartilages, has poor regenerative capacity and defects in the disc lie at the heart of many common TMJ disorders. This project seeks to understand the genetic identify of the TMJ disc and to create disc cells with reparative properties from human iPSCs.

Up to 70% of defects in the TMJ are due to displacement to the TMJ disc through trauma or wear and tear. This can lead to thinning or perforation of the disc, though these may also occur though trauma without disc displacement. In turn, disc displacement and damage is associated with osteoarthritis of the neighbouring condylar cartilage. Additionally, the disc cells can differentiate incorrectly, often after injury, losing their fibroblast morphology, leading to ossification/calcification of the disc, causing pain and limiting jaw movement. Calcification of the disc increases with age, and is, therefore, an increasing problem in an aging population.

The current surgical strategies including removal of the disc (discectomy) or joint reconstruction with autologous (typically from the ribs) or alloplastic materials. However, neither of these strategies is satisfactory. Therefore, disc repair is an important target for any future therapy, with early repair ideally preventing subsequent degeneration of the underlying cartilage.

In vivo, discs have a very poor ability to repair. In a rabbit in vivo perforation model no significant repair of holes was evident after 12 weeks. In addition, in this model the operated discs underwent ossification, suggesting that perforation had a negative consequence on cell fate in other parts of the disc. In keeping with this, disc cells in culture can undergo osteogenesis when cultured with BMPs.

In order to repair holes a tissue bioengineering approach has been taken in animal models. A variety of cell types supported on scaffolds have been incorporated into perforated discs with variable success at bridging the gap, largely because the cell type added is not always appropriate. The challenge is to create a tissue that does not undergo mineralisation and retains the disc's unique properties. These problems are exacerbated by the fact that we know very little about the cells that form the disc, their lineage and genetic signature, and how disc identity is regulated by molecular and mechanical signals. This project aims to address these key issues to enhance repair strategies.

During the project the student will investigate disc identify by comparing RNAseq signatures of human developing disc cells. Human tissue is available through the Human Developmental Biology Resource (HDBR), and the first supervisor, Prof Tucker, has a number of projects involving use of fresh embryonic and fetal craniofacial tissue from this resource.

This information will be used to tailor make disc cells from induced pluripotent stem cells. Human iPSC are available through resources such as the HiPSci (HipSci.org). The second supervisor, Prof Grigoriadis, is a in expert on differentiation of HiPSCs to make skeletal tissue for repair. The project will involve collaboration with clinical colleagues in the GSTT Oral and Maxillofacial Surgery group.

Project 23 - Artificial Intelligence to Improve Multiple Myeloma Assessment (AIMM)

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Abstract

Clinical background: Multiple myeloma is a debilitating bone marrow cancer arising from plasma cell transformation. Progression from monoclonal gammopathy of uncertain origin to myeloma is multifactorial, including genetic mutations & bone microenvironment changes; and heralded by non-specific bone pain. Patients present late, with complications e.g., pathological fractures, with a 5-year survival rate of 47%. NICE guidelines (NG35) advocate whole-body magnetic resonance imaging (MRI) in suspected/serum-confirmed myeloma as it is the most sensitive test for focal bone destruction (lytic lesions). But, with >14,000 images per MRI, clinical evaluation is time-consuming and subject to high observer variation. Artificial intelligence could improve clinical workflow & provide more objective assessment of disease status by automatically detecting focal lesions, quantifying whole-skeleton bone marrow replacement (MRI T1 Dixon fat-fraction) and plasma cell infiltration (MRI diffusion apparent diffusion co-efficient, ADC).

Aims:
mRES: To apply uncertainty-based deep neural networks for whole-body MRI segmentation to extract skeleton fat-fraction.
PhD: To develop novel whole-body imaging biomarkers using whole-body automatic segmentations, ADC and fat-fraction quantified from MRI; To evaluate performance of the new imaging biomarkers for myeloma in risk stratification for treatment and in monitoring treatment, alongside clinical, cytogenetic and pathologic markers.

Methods:
MRes project (3 months): A previously-developed pipeline based on a modified version of the well-known deep neural network 2D U-Net with uncertainty estimation applied to a cohort of 45 suspected myeloma patients (with reference standard radiologist-derived segmentations). Using the AI-segmented skeleton and the fat-fraction maps, the student will analyse the relations between marrow fat-fraction values and disease distribution at both whole-skeleton and anatomical region level.
PhD (3 years): This translational PhD will entail:
WP1: Optimization of uncertainty-based deep neural networks for whole-body MRI quantification of fat-fraction

Optimization of pipeline for accurately extracting fat-fraction to provide whole skeleton, anatomical region or lesional measures of bone marrow replacement.

WP2: Application of uncertainty-based deep neural networks for whole-body MRI quantification of ADC
Optimized pipeline applied and refined for diffusion MRI to detect focal disease and extract whole-skeleton ADC to provide whole skeleton, anatomical region or lesional measures of plasma cell infiltration. Relation between ADC and disease status, including bone biopsy will be assessed.

WP3: Developing novel biomarkers to detect, stage and monitor disease.

Biomarkers based on combining imaging and clinicopathological data will be developed. E.g., segmented fat-fraction and ADC maps combined as input to a deep neural network architecture. Classification of the disease will be at both patient and regional levels.

Data: Approved access to >500 MRIs from >200 patients for training, validation and testing. All the pipelines will be tested on a temporally separated cohort of 35 on-treatment patients with serial MRI to study the generalizability of proposed approaches.

Likely impact: By automating assessment & facilitating clinical workflow, AI-enabled evaluation has the potential to transform imaging practice. By providing more objective assessment of disease status, this AI project will improve disease detection, risk stratification & monitoring of disease progression, and ultimately outcomes through earlier initiation &/or switching of therapies. This approach is translatable to other cancer types e.g., breast, prostate.

Project 24 - Cross talk between the innate immune system and the vaginal microbiota – understanding how the vaginal environment modifies risk of preterm birth

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Abstract
Preterm labour accounts for approximately two thirds of all preterm births and the associated morbidity and mortality. The biological pathways leading to the spontaneous onset of preterm labour are unclear although there is a strong association between an inflammatory cervicovaginal environment, shortening of the cervix in the mid trimester, and risk of preterm birth. The cervicovaginal environment is shaped both by the resident microbiota and local inflammation driven by the host response (epithelia, immune cells and mucous). The contributions of the microbiota, metabolome and host defence peptides have been investigated, but less is known about the immune cell populations and how they may respond to the vaginal environment. This study plans to investigate the maternal immune cell population at the cervicovaginal interface in early to mid-pregnancy (10-24 weeks of gestation). Our preliminary study indicates that neutrophils and then monocytes are the predominant cell type. Using cell-type enriched RNAseq, we have begun to characterise associations between the cervical neutrophil transcriptome and the cervicovaginal metagenome. Microbial diversity impacts on the neutrophil transcriptome and sub-population phenotype. Genes involved in neutrophil mediated immunity, activation, degranulation, and other immune functions correlate negatively with Gardnerella vaginalis abundance and positively with Lactobacillus iners abundance; with both microbes previously associated with poor birth outcome. This initial work has established our rationale for investigating associations between the innate immune response, cervical shortening and birth outcome.

We hypothesise that there is a bidirectional influence of the vaginal environment and the immune cell response. Differences in host response will impact on cervical integrity and hence risk of preterm birth. Using a computational systems biology approach will integrate signals (metagenomics, transcriptomics, flow cytometry/mass cytometry and proteomics) to determine cross talk between components of the innate immune system and different vaginal environments associated with term (n=100) and preterm birth (n=50 preterm). In paired cervical and maternal blood samples, the student will correlate the local tissue and circulating immune cell transcriptomes with the aim to identify a blood signature that is more clinically accessible, as well as identifying novel targets to ameliorate the risk of preterm birth. In addition, there will be in vitro studies to assess how challenge of maternal immune cells with specific bacterial products/strains influences neutrophil priming and function. This study will recruit women from general antenatal clinics as

well our Preterm Surveillance Clinic under the ongoing INSIGHT cohort study ethics. It will utilise immunological expertise of Dr Gibbons lab and the BRC Flow cytometry core. Nextgen sequencing will be undertaken in collaboration with the BRC genomics/sequencing core. Bioinformatics/statistics training supported by the BRC Core, Dr Mario Falchi's team (Twins) and an ongoing collaboration with University of Stanford, California, USA.

Project 25 - Progressing candidate biomarkers of response to biologics in psoriasis onto the biomarker development pipeline

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Abstract

Psoriasis is an immune mediated inflammatory disease of the skin, affecting 2% of UK adults. It is characterized by disfiguring and stigmatizing lesions that can be itchy or painful and severely affect patient' quality of life. Proinflammatory cytokines tumor necrosis factor (TNF), interleukin-23 (IL-23) and interleukin-17A (IL-17A), drive disease pathogenesis and are the molecular target of the biological therapies ("biologics) used to treat moderate-to severe cases. Twelve biologics are currently licensed for psoriasis and have transformed disease management and patient' lives, but they are expensive and clinical response is not adequate in every patient. With different therapeutic agents available in psoriasis, there is scope to implement a precision medicine approach, whereby each patient receives the most effective therapy for their condition.

Biological markers ("biomarkers") are measurable characteristics indicating normal or pathogenic processes or responses to an intervention. Specifically, predictive biomarkers distinguish and stratify individuals based on their response to a medical intervention. No predictive biomarker of response to biologics is currently used to guide prescription in psoriasis, as none of the candidates identified so far has been validated in clinical trials. Thus, there is an unmet clinical need to progress candidate biomarkers onto the developmental pipeline and towards clinical implementation. Our group is engaged in a nation-wide biomarker development program aimed at identifying, validating and ultimately implementing blood-borne predictive biomarker of response in psoriasis. We have recently identified, replicated, and mechanistically validated, NF-□Bp65 phosphorylation in type-2 conventional dendritic cells (cDC2) as a baseline predictive biomarker of response to the commonly prescribed anti-TNF biologic adalimumab (Andres-Ejarque et al., Nat Commun 2021). The next step is to develop our existing assay, which requires isolation of peripheral blood mononuclear cells (PBMC), into a simpler test using whole blood without the need for PBMCs isolation. Such simplified test would be more easily implemented in a clinical setting for validation in clinical trials. Additional ongoing work in our lab has also uncovered putative blood-borne biomarkers of response to the anti-IL-12/23p40 ustekinumab and anti IL-17A secukinumab which require replication in independent cohorts and further investigation to move forward onto the biomaker development pipeline.

Thus, the aims of this project are:

1) to develop a phosphoflow cytometry test to measure LPS-induced NF-kBp65 phosphorylation in cDC2 in fresh whole blood of healthy volunteeers (in the rotation phase).

2) to progress one of the candidate biomarkers of response to either ustekinumab or secukinumab on the biomaker development pipeline by performing replication in independent cohorts and mechanistic investigations (in the 3 years of the PhD).

The project will use cutting-edge advanced technologies (e.g. imaging and spectral flow cytometry, imaging mass cytometry, special transcriptomic, single cell RNA-sequencing) coupled with high dimensional data analysis, bioinformatics and statistics, in which the student will receive training, alongside training for complementary and transferable skills.

Taken together, this project will provide a solid and multidisciplinary translational research experience, with the opportunity to create a concrete and real benefit for patients with psoriasis through the progression of candidate predictive biomarkers on the path towards clinical implementation.

Project 26 - Multi-omics to understand the genetic and molecular mechanisms of Type 2 Diabetes subtypes

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Abstract

Type 2 Diabetes and obesity-related traits are global epidemics. In the UK alone, ~4 million people are living with diabetes and 10% of the NHS budget is spent on diabetes. Understanding the molecular mechanisms linked to genetic risk of diabetes will help direct novel treatments and aid prevention. A current limitation to early detection and treatment of Type 2 Diabetes is its diverse and heterogenous clinical presentation. The age of onset varies widely, individuals can exhibit a range of clinical characteristics, disease progression trajectories and response to medication. Improved methods for early detection and stratification of patients would have a large clinical impact.

Genome wide association studies (GWAS) of large biobanks have identified hundreds of genetic loci associated with risk of Type 2 Diabetes, and there is much excitement in using the resultant Polygenetic Risk Scores (PRS) from these studies to predict risk of Type 2 Diabetes from an individual's genetic data. However, the accuracy of Type 2 Diabetes Polygenetic Risk Scores has been limited, potentially due to the heterogenous nature of the disease. Several studies have recently identified five subtypes of Type 2 Diabetes with differing genetic risk profiles and phenotypic presentation (For example Aly et al, Nature Genetics 2021). These studies suggest that the underlying molecular drivers of disease may differ across the genetic subtypes resulting in differential ability of commonly used diagnostics to detect and treat early signs of disease across the subtypes.

This project will leverage large genotyped biobanks and deep 'omic profiling to 1) identify the molecular signature of the Type 2 Diabetes genetic subtypes and 2) determine if the Type 2 Diabetes subtype Polygenetic Risk Scores predictive ability can be augmented by combining genetic and 'omic data. The association between Type 2 Diabetes Polygenetic Risk Scores and 'omic profiles will first be investigated in the TwinsUK cohort which is located at King's College London. This includes an unparalled dataset of cross-sectional and longitudinal 'omics, including deep tissue transcriptomics from adipose, skin, gut and blood, and epigenetics, proteomics, metabolomics, and microbiome. The project will also leverage large external genomic datasets. Identifying the 'omic signature of each sub type will elucidate the molecular mechanisms underlying disease risk and progression. Secondly, the project will utilize Electronic Health Records from TwinsUK and other datasets to determine if the combination of genetic and 'omic data can better predict future development of disease or disease progression. This will include incorporating both 'omic data at baseline and trajectories over time using computational methodologies.

Project 27 - Translation towards a first in human gene therapy for Spinal Cord Injury

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Abstract
This is an exciting time for translating experimental Advanced Therapies into treatments for spinal cord injury (SCI). There are currently no regenerative therapies available. Over the last 5 years we have developed a viable gene therapy approach for treating SCI in pre-clinical models. We are on the cusp of translating this therapy to first-in-human studies. We are leading a programme to establish a world-first regenerative gene therapy for traumatic SCIs affecting upper limb mobility. Advanced gene therapy technology has been employed to enable stable and long-term delivery of matrix-modifying enzymes which digest pathological scar tissue and enable engagement of dormant motor pathways and reconnection with target muscles. This results in recovery of upper limb mobility and skilled hand function in advanced pre-clinical studies. We are initiating the production of GMP grade vectors. Our team will develop an innovative trial design involving neurosurgical delivery of the gene therapy into the spinal cord, followed by specialist neurorehabilitation.

Aims

1) Evaluate a spinal cord assessment tool using transcranial magnetic stimulation

For a clinical trial to have the best chance of success, we need to address critical steps which include validation of assessment plans and delivery methods of the gene therapy. Following two successful PPI events with our rehabilitation partners, the student will recruit chronic SCI subjects to carry out European Multicenter Study about SCI (EM-SCI) assessments. EM-SCI assessment tools are well established assessment tools for SCI patients and include the EM-SCI 'core' assessments (ISNCSCI, WISCI-III, Walk Test, SCIM) and EM-SCI 'additional' assessments (GRASSP, Pain score, neurophysiology). The student will also validate and compare Transcranial Magnetic Stimulation (TMS) with EM-SCI assessments in stable chronic SCI subjects. TMS with combined Tractography MRI of the cortico-spinal tract (a major motor pathway critical for voluntary movement) will allow functional characterisation and density measurements of this tract in SCI subjects compared to control subjects.

2) Validate a novel vector delivery system in cadaveric human spinal cord

A second requirement before a first-in-human trial is validation of the delivery technology of the gene therapy. Accurate delivery of microlitre volumes of the gene therapy into the spinal cord is critical for trial

success. Our commercial partners, ClearPoint, are developing a novel vector delivery system ensuring accurate site and volume delivery which requires validation. In this project, the student will use a state-of-the-art surgical validation suite including a mock operating room (within Biomedical Engineering and Imaging Sciences at St. Thomas') to simulate delivery into cadaveric human spinal cord. This will allow ClearPoint to obtain regulatory approval for use of the system for our gene therapy trial in addition to validating accurate delivery of the product.

3) Develop a Phase 1/2 clinical trial

The student will develop the trial protocol prior to submission to the MHRA. This includes incorporation of the findings from Aim 1 and Aim 2 above, continued PPI input, and development of an adaptive trial design for a first-in-human Phase1/2 trial. The first of 12 patients will be recruited during this PhD.

Project 28 - Elucidating the mechanisms of hepatocyte regeneration in humans

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Abstract

Background
Advances in surgical oncology have revolutionised the treatment of primary and secondary liver malignancies, meaning that more patients are able to be treated with liver resections. Techniques such as portal vein embolization stimulate growth of the future liver remnant and allow patients with large and anatomically challenging tumours to be considered for surgery. However, with this comes the significant risk of the remnant liver being functionally inadequate, resulting in life-threatening liver failure due to small-for-size complications. We currently lack objective and quantifiable methods to predict outcomes and risk stratify these patients. An important and unmet clinical need therefore is the ability to accurately predict the functional reserve and regenerative capacity of a patient's liver. This will lead to benefits in other related fields of liver surgery, for example transplantation. The inevitable ischaemic reperfusion injury and inflammatory response which occurs in the immediate post-transplant period contributes to a huge stress for the new liver which sometimes manifests as primary non-function. The resilience of such a liver to withstand these inflammatory injuries will be linked to pre-existing organ function.

Animal models have shown that liver regeneration following injury involve the Wnt pathway and result in expansion of specific zonal hepatocytes. However, whether these mechanisms are the same in human liver remains a major gap in knowledge. Our previous work on chronic liver disease has identified novel driver mutations such as FOXO1 which allow human hepatocytes to proliferate and form large clonal expansions. It is unknown whether these pathways are active in healthy livers and provide proliferative and survival cues to hepatocytes. We hypothesise that livers which contain driver mutations regenerate more successfully which correlates to better clinical outcomes. To investigate this, the project will utilise cutting edge genomic sequencing technologies, CRISPR gene editing and in vitro models using organoids and induced pluripotent stem cells (iPSCs).

Methods:
The effects of the driver mutations on hepatocytes will first be investigated by generating CRISPR gene knockouts of driver mutations in iPSCs. These will then be differentiated into liver organoids in vitro and characterised by immunostaining, flow cytometry, quantifying the growth kinetics through single cell clonal assays and performing RNA sequencing. Next, biopsies of regenerating livers (typically the left lobe) will be taken at the time of resection surgery in order to capture novel populations of proliferating hepatocytes. Half of the tissue will be processed and analysed for single cell RNA sequencing. The other half will be used to generate primary patient-derived liver organoids using established protocols. The presence of the driver mutations in the patient derived organoids will be assessed by whole exome sequencing and compared to those described in the context of chronic liver disease. Finally, the Kings liver biobank will be interrogated and biopsies sent for exome sequencing. The presence of driver mutations identified will then be correlated with known clinical outcomes such as post-operative liver dysfunction to reach a predictive score in order to stratify patients undergoing liver resection and liver transplants.

Project 29 - Towards personalised medicine approaches to IVF treatment regimens

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Abstract

Background.

Infertility is a life changing disorder, with 1 in 7 couples in the UK estimated to experience difficulties conceiving. NICE recommends IVF as the effective treatment for prolonged unresolved infertility, however despite several technological advances, IVF success rates have remained relatively unchanged over the last decade and low, at ~26% live birth rate per treatment cycle in the UK for combined age groups. Advanced age (<35), which is a key indicator of fertility, is the fastest growing group of patients seeking fertility treatment, with the average age of women increasing from 32 in 1991 to 35.7 in 2019. Yet, the success rate of IVF decreases dramatically with age, highlighting the need for refinements in clinical management strategies for these patients.

The coordinated actions of the gonadotrophin hormones, follicle stimulating hormone (FSH) and luteinising hormone (LH) support ovarian follicle steroid hormone production, somatic granulosa cell growth, and oocyte maturation. During the ovarian stimulation phase of IVF, multiple ovarian follicles are recruited by the administration of either recombinant FSH variants (FSH biosimilars) or menopausal gonadotrophins (hMGs). Despite published studies by us and others identifying differences in the in vitro functional properties of these gonadotrophin hormones, little is known about how differing FSH biosimilars and hMGs impact ovarian function and IVF outcomes in women of young and advancing age. Indeed, recent ESHRE guidelines on IVF ovarian stimulation have identified this as a knowledge gap, identifying an unmet need to understand how advanced age impacts responses to ovarian stimulation drugs, to identify which may provide the best treatment regimen and move to a personalised medicine approach to IVF.

Overall aim

This PhD project aims to investigate how ageing impacts the ovarian transcriptome during IVF and how cellular responses to ovarian stimulation drugs are modulated by age and how this correlates with IVF outcomes.

Methods

Ethics: IRAS 264510 with recruitment from GSTT and KCH.

Inclusion criteria: women aged 18-45 undergoing routine IVF.

Experimental outline: Granulosa cells will be harvested from follicular aspirates generated at the time of oocyte retrieval. Isolated granulosa cells will be RNA extracted and subjected to RNAseq analysis. Samples will be stratified by the following age groupings (<35, 35-37, 38-39, 40-42, 43+), n=4, with samples obtained from patients with matched antral follicle counts, to minimise ovarian reserve as a confounder. Key pathways/targets that are differentially modulated by age will probed in further patient samples that have been stimulated -/+ FSH biosimilars and hMGs, and gene expression changes correlated with IVF outcome measures (oocyte number, quality, day 3/5 embryos, pregnancy, live birth). Knockdown and over-expression studies in cultured granulosa cells of RNAseq identified pathways will provide functional assessment of gene roles.

Outcomes

Identification of key genes/pathways that are modulated by age and IVF hormonal regimen, and correlation with IVF outcomes. These data will provide a pathway towards stratifying IVF hormonal treatment regimens based on age to move to a personalised medicine approach to ovarian stimulation. Moreover, these data may highlight novel therapeutic targets to improve IVF success rates.

Project 30 - Sex-specific differences in the genomic architecture of acne to drive drug target discovery and therapeutic regimes

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Abstract
Acne vulgaris is the most prevalent skin disease worldwide affecting up to 85% of teenagers, with 8% having severe disease. Acne lesions result from inflammation of pilosebaceous units in the skin of the face, neck, chest and back. In addition to inflammatory pain and discomfort, as a visible skin condition, the symptoms and scarring are associated with a substantial psychological burden. The widespread media portrayal of body image and appearance leaves teenagers vulnerable to visible changes on their skin as they establish their social identity. The negative impact of acne on psychological wellbeing is evidenced by strong associations with emotional stress and neuropsychiatric disturbances including depression, suicidal ideation and suicide. The identification of genetic mechanisms underlying disease is an established route to unravelling causal biological processes, from which therapies can be optimally designed to intervene. Our most recent genome wide meta-analysis represents the largest genetic study of acne ever undertaken (Mitchell et al Nature Commun 2022), comprising 20,165 cases of European ancestry from nine independent cohorts. Association testing led to the identification of 43 regions of the genome at which genetic variation contributes to an individuals' risk of developing acne. Critically, the polygenic burden is highly correlated with self-reported disease severity. This observation is consistent with more severe symptoms are associated with increased genetic risk.

Acne is sexually dimorphic; females typically experience greater fluctuation in symptoms, often with a characteristic perimenstrual flare. We have previously investigated sex specific genetic effects in acne, identifying a significant difference in the effect size a coding variant in WNT10A has on acne risk between males and females. We hypothesise that sex bias in acne risk is prevalent at other loci in the genome.
The proposed project will investigate sex specific differences in the genetic risk of acne using genome wide genetic variation data. The male and female cohorts will each comprise >20,000 cases, which represents a major opportunity for novel discovery over our previous sex stratified analysis of 1,670 male and 2,153 female cases (Petridis et al Nature Commun 2018). Sex specific effects identified in the first phase of the project will be used as a substate to computational genomic approaches to identify genetically supported therapeutic hypotheses with relevance to males and females. These large-scale genetic investigations of sex specific acne risk will facilitate the evaluation of the effectiveness of lifestyle modifications of acne in both males and females using Mendelian Randomisation methodologies.

Project 31 - Validation of serum levels of soluble OX40 and OX40L in predicting disease flares in lupus?

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Abstract
Systemic Lupus Erythematosus is a chronic, multi-system autoimmune disease. There are more females than males with lupus and increased disease severity in non-European ancestries. The relapsing-remitting nature of the disease course poses challenges for treatment and exceptionally hard to live with as a patient. Furthermore, there are no reliable biomarkers to predict flare-ups of the disease. Development of biomarkers for disease flares may allow more rapid adjustment/change of drug treatments, resulting in less severe flares and greater sense of control for patients in their daily lives.

Two such potential biomarkers are the lymphocyte costimulatory molecules, OX40 and OX40L. OX40 is the unique receptor for OX40L and both molecules are from the TNF superfamily of receptors. We have shown that genetic variation at these two risk factors for lupus leads to increased gene expression. Our hypothesis is that elevated expression of one or both risk factors is correlated with increased disease activity.
We have preliminary data in 20 patients revealing that 20% individuals had elevated serum levels of OX40L and/or OX40, using the cutting-edge Meso-Scale multiplex assays from MSD to assay serum protein levels. The 20% patients with elevated serum OX40/OX40L have more active disease. Therefore, in this PhD project we will both increase our cohort size and take additional samples from patients over a longer time-period, to provide stronger validation that OX40/OX40L are biomarkers of disease flares.

The aims of the project are to: 1) Validate the predictive capacity of serum OX40 and OX40L levels for disease flare, by tracking serum levels of these two proteins from lupus patients over a two-year period. We will employ cutting-edge Meso-Scale multiplex assays from MSD to assess protein levels. 2) Ascertain the likely cellular source of serum OX40 and OX40L, by correlating the serum protein levels with the cell-surface expression using flow-cytometry, in blood samples from patients attending clinic during a flare with a repeat sample after resolution of the peak of the flare. We will determine whether the pattern of membrane-bound OX40 and OX40L changes on specific immune cell-types relative to levels of serum OX40 and OX40L. 3) Develop a Digital Patient Engagement App (part of an ongoing collaboration with Cambridge Digital Health Ltd) to monitor self-reported symptoms between clinic visits. Patients input symptom information via a phone or computer application. The data will be anonymised automatically and uploaded to a secure GPDR-compliant database behind the university firewall. Plotting symptom progression over time will allow identification of individuals who have a potential flare-up of their symptoms. 4) Assess the feasibility of large-scale biomarker monitoring between clinic visits using self-administered finger-prick tests, involving participants sending in bi-weekly finger-prick blood samples for biomarker assay.

Project 34 - Novel oncogenic mechanisms: regulation of physical forces to protect squeezed nuclei

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Abstract
Cancer metastasis, i.e., the dissemination of cancer cells away from a primary tumour and colonisation at distal sites, accounts for most cancer-related deaths. Thus, there is an urgent need to understand how metastatic potential is acquired. To invade surrounding tissues, cancer cells need to migrate through dense and constricted spaces, which results in high mechanical stress on the nucleus, a large and stiff organelle. This can lead to nuclear envelope ruptures during interphase (NERDI), the transient mixing of cytoplasmic and nuclear contents and, in turn, DNA damage. Although NERDIs are rapidly repaired, the acquired DNA damage causes genome instability, further enhancing the cell's metastatic potential.

Recent studies have identified the endosomal sorting complex required for transport (ESCRT) machinery as a key membrane remodelling pathway required for nuclear envelope (NE) resealing after NERDI. Importantly, we have shown that BROX, an ESCRT-associated protein, promotes the relaxation of cytoskeleton-mediated compressive forces on the nuclear surface, thereby facilitating efficient membrane resealing and protecting the genome from damage1. At a mechanistic level, BROX regulates NE homeostasis by restricting the cytoskeletal forces imposed by Nesprin-2G, a protein that transfers mechanical forces from the cytoskeleton to the nucleus1. Therefore, BROX silencing destabilizes the delicate mechanical balance of the NE, impairing efficient NERDI repair, ultimately compromising genome stability. Critically haploinsufficiency in the BROX gene has been associated with hereditary thyroid cancer, suggesting that mechanical dysregulation at the NE may act as a novel oncogenic mechanism.

Building on these findings, we propose that BROX is part of a novel mechanism that prevents genomic instability by regulating mechanical forces at the NE, thus preventing cell transformation and cancer development. To test this hypothesis, this project will explore whether mechanoregulation at the NE is disturbed in cells bearing BROX mutations found in thyroid cancer. In addition, we have identified a small number of individuals with predicted loss-of-function and missense variants in BROX in the Genes and Health study, a cohort of Pakistani and Bangladeshi individuals with high levels of consanguinity and therefore enriched for naturally occurring variants.

Aim1. The student will determine the effect of the BROX genetic variants described above in mechanical regulation at the NE. Gene editing of model cell lines will generate cell lines expressing the relevant BROX

variants. The effect of these variants in NERDI repair and genomic stability will be determined by cutting edge microscopy. Biophysical techniques, such as atomic force microscopy, will be performed in collaboration with the Physics department to determine the mechanical properties of the NE in these cells.

Aim2. The student will establish whether mechanoregulation at the NE is disturbed in cells derived from BROX deficient individuals identified in the Genes and Health Cohort. Briefly, these primary cells will be studied by microscopy and biophysical approaches as described for Aim1, to determine the mechanical properties of the nuclei and the accumulation of genetic damage.

References: 1) Wallis, Ventimiglia, Otigbah et al. “The ESCRT machinery counteracts Nesprin-2G-mediated mechanical forces during nuclear envelope repair.” Dev Cell (2021)

Project 35 - Mesenchymal stem cell modulation of the aged environment in chronic limb threatening ischaemia

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Abstract
Peripheral arterial disease affects 20% of patients over 60yrs in the UK with ~11% developing chronic limb threatening ischaemia (CLTI). More CLTI patients die from a cardiovascular cause within 5 years than with any type of cancer expect lung. The high incidence of CLTI in combination with its highly fatal course from major limb amputation and/or cardiac disease heart failure/myocardial infarction) make this condition a major under-recognised threat to public health.

Patients with CLTI have elevated levels of inflammatory proteins in their serum; this is independently associated with major cardiovascular events. Chronic inflammation and ageing contribute to the pathogenesis of cardiovascular disease. “Inflammaging” is a chronic, sterile, pro-inflammatory status that occurs during ageing. Senescent cells, which accumulate in aged and diseased tissues, secrete pro-inflammatory senescence associated-secretory phenotype (SASP) factors. Ageing results in immunosenescence and a cycle of further activation of inflammatory pathways, including reactive oxygen species/matrix metalloproteinases that are involved in dysfunctional cardiac and vascular remodelling. Mesenchymal stromal cells (MSCs) have a powerful immunomodulatory and anti-inflammatory function. We have shown that exposure of cardiomyocytes or human microvascular endothelial cells to pro-inflammatory SASP factors/proteins decreases cell viability, proliferation and angiogenesis and that this is rescued by treatment with MSCs. Delivery of MSCs to the ischaemic murine limb results in reduced inflammatory cell content, modulation of infiltrating monocytes towards an anti-inflammatory phenotype and significant improvement in revascularisation and limb salvage.

With our translational bench-to-bedside programme of work in the BRC at GSTT/KCL, we have generated of a bank of clinical grade MSCs (MSC001). This advanced therapy has the potential for use in CLTI patients to modulate inflammaging.

We hypothesise that:

1. MSCs modulate inflammation and attenuate the damaging effects of inflammation to the cardiovascular system.

2. Inflammaging and the SASP is a central process in dysfunctional tissue remodelling seen in elderly patients with chronic limb threatening ischemia (CLTI) and this process can be modulated with MSCs to revascularise ischaemic tissue.

Objective 1. To determine whether MSC001 can attenuate the damaging effects of inflammation on the cardiovascular system.

Human cardiomyocytes, vascular endothelial cells and fibroblasts will be stimulated with aged and CLTI serum and co-cultured with and without MSC001. We hypothesise that the MSC001 product will rescue cell senescence and death.

Objective 2: Characterising senescence and the SASP in healthy and ischaemic muscle from patients with CLTI. Blood and muscle from young and old patients with CLTI as well as matched controls will be analysed for cell senescence/SASP, immune cell senescence and M1/M2 macrophage polarisation. We hypothesise that the muscle tissue from older patients will be higher in cell senescence, immunosenescence, SASP content and ratio of M1:M2 macrophages.

Objective 3: Modulating the tissue environment with MSCs using in vitro bio-assays
Human endothelial cells and or monocytes will be cultured with serum and conditioned media generated from ischaemic muscle biopsies from young and old patients with CLTI. We hypothesise that addition of MSC001 to this system will rescue the deleterious effects of the pro-inflammatory tissue environment on endothelial and monocyte cell function and phenotype.

Project 36 - From genotype to phenotype: characterising the genes that determine bone fragility and fracture risk

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Abstract
Osteoporosis is a leading cause of death and disability; excepting COVID-19, osteoporotic fracture is the commonest cause for women over 40 to be hospitalised. The great unmet need in osteoporosis therapeutics is for agents that stimulate new bone formation ("anabolics"). Currently there is only one anabolic agent licensed in the UK (one other licensed in the US); both are injectables, limiting their use, and no oral anabolic agent exists. Genome-wide association studies have already identified over 500 genetic variants associated with bone density; however, for most variants the mechanism by which they influence bone density and osteoporosis risk, and the cell type(s) involved, are unknown. The main cells in bone are osteoclasts (responsible for bone resorption), osteoblasts (bone formation) and osteocytes (bone regulation, derived from osteoblasts). Variants that affect osteoblast and osteocyte function are of particular interest, as exploiting these pathways may enhance bone formation.

This project aims to identify potential anabolic (bone-forming) therapeutic targets for treating osteoporosis, using induced pluripotent stem cells [iPSCs] to assess the functional consequences of pathogenic variants in genetic loci known to be associated with bone density variation, and takes a novel approach to functional genomics: going from genotype to phenotype: assessing both clinical and cellular repercussions of carriage of pathogenic variants, and working in populations with diverse ethnicities.

The East London Genes and Health study (ELGH, now combined with Born in Bradford [BiB] as Genes&Health) is one of the world's largest community-based genetic studies. ELGH has already identified over 5000 individuals at high risk of homozygosity for functional genetic variants, due to parental relatedness; recent funding has enabled extensive further sequencing. Similarly, BiB is also currently sequencing their large population, also enriched with individuals carrying homozygous variants . We will work with these cohorts to identify participants carrying variants predicted to affect genes in loci known to be associated with bone density and/or fracture. These participants will then be clinically phenotyped, including measurement of bone density, bone turnover, and calcium/phosphate homeostasis, demonstrating functional consequence of homozygous carriage of pathogenic/likely pathogenic variants. Where available, the obligate heterozygote parents and any available siblings will also be phenotyped. Importantly, we do not assume the direction of effect on bone, noting here that pathogenic variants in LRP5, genetic studies of which informed the development of romosozumab, can cause both high and low bone density.

iPSCs will then be produced from those individuals, and transformed into the three stem cell lines (endoderm, mesoderm, ectoderm) before differentiation into osteoclasts and osteoblasts, with characterisation of variant effects on cellular function (discussed in more detail below). In particular, we

aim to identify variants that influence the function of osteoblasts, the bone forming cells, as a means of identifying potential anabolic genes and processes. Once identified, studies will then focus on determining the exact functional mechanisms by which these variants influence bone density and fracture propensity, and how these may be exploited to translate these findings into novel therapeutics for osteoporosis.

Project 37 - Pathophysiology of widespread pain and fatigue in psoriatic arthritis

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Abstract
Fibromyalgia syndrome (FMS) is an incurable condition characterized by chronic widespread pain, fatigue, mood disorders, anxiety, and stress. Despite that over 2% (>80% of which are women) of the population has fibromyalgia, the causes, and mechanisms responsible have remained unknown and no effective drugs or diagnostic tests are available. Currently, most researchers believe that FMS originates in the brain, as a consequence of aberrant sensory processing. However, recent work from Andersson and colleagues has revealed that severe fibromyalgia (in patients without other rheumatic or autoimmune conditions) is an autoantibody mediated condition, in which pain-sensing neurons are hyperexcitable and the laboratory is now exploring the autoimmune and neuronal mechanisms responsible for pain and fatigue in FMS. Administration of immunoglobulin G (IgG) from FMS patients to mice recapitulated multiple symptoms of the condition in mice, such as painful sensory hypersensitivities, small fibre pathology (neuropathy), reduced activity, reduced muscle strength, and sensitization of nociceptive afferents. Importantly, patient IgG bound to cells in mice and human dorsal root ganglia. These observations comprehensively fulfil Witebsky's postulates for identification of an autoimmune disease and will transform future research and management of FMS.

The prevalence of FMS is markedly higher (around 20-30%) in patients diagnosed with rheumatic diseases than in the general population (~2.5%). Here, we aim to determine whether FMS with well-controlled psoriatic arthritis (by conventional disease modifying antirheumatic drugs, DMARDs, or biologic therapies) shares the autoimmune pathophysiology recently revealed for "primary" FMS. Patients with and without fibromyalgia will be recruited and phenotyped with a focus on their inflammation, pain, and fatigue. Peripheral blood mononuclear cells (PBMCs) will be collected and analysed to determine whether the presence of fibromyalgia is associated with an altered distribution or number of immune cells, using the Flow Cytometry Platform in the BRC. Serum will be collected to evaluate the presence of autoreactive IgG and to assess whether the profiles of reactivities differ between patients with and without FMS. We will also measure the serum concentration of a panel of cytokines and chemokines using the Luminex analyser in Guy's BRC. In collaboration with Karolinska Institutet, we will perform an exhaustive metabolomic analysis of sera to identify biomarkers that associate specifically with pain and fatigue in this group of patients. We will use passive transfer of IgG from patients to mice to determine whether IgG from rheumatic patients with and without FMS is responsible for pain or other symptoms and signs, similar to our findings with samples

from patients with “primary” FMS (this line of investigation is funded independently). This studentship thus presents a rare opportunity to discover mechanisms responsible for a common syndrome, and to identify causes of widespread pain and fatigue in psoriatic arthritis. Despite the remarkable improvements in the clinical management of rheumatic diseases over the last couple of decades, pain and fatigue remain major challenges for patients, suggesting that these symptoms are not caused by the inflammatory processes responsible for rheumatic diseases. The proposed studies are thus likely to significantly improve our understanding of comorbid FMS.

Project 38 - Artificial intelligence for diagnosis of critical brain pathology using neuroimaging

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Abstract

Research Question: We hypothesise that in adult patients suspected to have a brain tumour, automated tools can (i) accurately stratify patients into those who require brain scan imaging in a primary care setting and (ii) accurately diagnose and report brain tumours in a secondary/tertiary care setting (using CT or MRI).

Background: A clinically validated tool that identifies brain tumours during brain CT or MRI scans does not exist. This project will meet this clinical need by providing a deep learning model that can automate the identification of brain tumours immediately in 'real-world' conditions – and produce a report. This is important as over 330,000 patients are waiting more than 30 days for their reports in the UK. This number is forecast to increase, as there is a greater demand for CT and MRI than there is availability of radiologists to report these scans. UK-specific workforce shortages in clinical radiology are negatively impacting patient care by delaying diagnosis; a similar picture is seen globally. Immediate identification of a brain tumour potentially allows early intervention to improve short- and long term clinical outcomes. In general, early diagnosis would result in lower costs for the healthcare system because less specialist medicine and fewer hours of treatment are needed for the patient to recover. Given the huge demand in scan referrals and given that only 1.4% of suspected patients referred by their GP for scanning will have a brain tumour, stratification is also essential. Clinical features can help stratification, but other available investigation that the GP has access to, will also be helpful in a prediction model.

Objectives: The aim of this project is to develop a tool that identifies critical abnormalities on magnetic resonance imaging (MRI) brain scans using deep learning. The objectives are: 1. Build a classification model to stratify patients with undiscovered brain tumours for brain scan imaging. 2. Build a classification model to determine brain tumours from brain scans. 3. Clean datasets for patient groups where symptom key words may improve classification accuracy. 4. Validation of the classification model using routine clinical and imaging data. 5. To give a personalized output regarding the likelihood (including confidence intervals) and location of a brain tumour. 6. To assess clinical use for brain tumour reporting and flagging of scans

Project 39 - Identifying key mechanisms that maintain anxiety and depression in pregnant women and new mothers and conducting a pilot study of a new pregnancy tailored intervention to prevent perinatal anxiety and depression

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Abstract
One-in-four pregnant women report mental-health problems, most commonly depression and anxiety. This is associated with multiple significant adverse consequences for these women, their unborn child, children and partners, including reduced responsivity to babies (Stein et al., 2012), impairments in childhood development, and a two-fold increase in risk of a child developing psychological disorders (O'Donnell et al., 2014).

We need to understand the key mechanisms that predict and maintain perinatal anxiety and depression. We also need to develop more scalable, accessible interventions to prevent perinatal anxiety and depression. Worry about the future and mulling over negative events from the past (rumination) are forms of repetitive negative thinking which predicts later levels of anxiety and depression in non-pregnant populations. Repetitive negative thinking, as well as anxiety and depression once established, are all maintained by unhelpful cognitive processes such as the tendency to focus on attention on negative information, interpret unclear or uncertain information in negative ways, as well as difficulty deliberately shifting focus away from negative streams of thinking (Hirsch & Mathews 2012). We need to establish whether similar mechanisms maintain perinatal repetitive negative thinking, and therefore increase the risk of perinatal anxiety and depression, since they can then form targets for new prevention interventions for perinatal anxiety and depression. Our work with PPIE indicates that any perinatal anxiety/depression prevention intervention should be offered while the women are pregnant, since women with experience of perinatal anxiety and depression think that this is a more feasible time to complete an intervention, as well as providing an opportunity to prevent perinatal anxiety and depression.

This PhD will provide an exciting opportunity to be at the cutting edge of cognitive research into perinatal repetitive negative thinking, and prevention of perinatal anxiety and depression. The PhD will involve an experimental study that will examine cognitive mechanisms associated with repetitive negative thinking, anxiety and depression in other populations. These processes will be assessed using pregnancy tailored experimental materials to make them pertinent to the women's current experience. We will determine whether the cognitive processes are associated with repetitive negative thinking in pregnant women and if they also help predict later levels of perinatal anxiety and depression.

The second phase of the PhD will involve adaptation of a web-based repetitive negative thinking intervention designed to target unhelpful cognitive processes and reduce later levels of anxiety and depression. We will work with women with lived experience of perinatal anxiety and depression to tailor it the day-to-day lives of

pregnant women. We will then conduct a pilot study to determine whether adding the web-based intervention to usual maternity care can help prevent subsequent anxiety and depression two-months later. Pregnant women with high levels of repetitive negative thinking will be randomised after baseline assessment to usual maternity care plus the one-month web-based intervention or usual maternity care alone.

Project 44 - Using machine-learning to predict pre-symptomatic intestinal inflammation in Crohn's disease

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Abstract

We are proposing a novel approach to allow early-detection and diagnosis of Crohn's Disease (CD) in the younger relatives of patients who are not yet showing any symptoms. This is because biologics medication in very early CD has been shown to significantly improve therapeutic response, increase the duration of disease remission, and reduce the long-term complications. Some groups of individuals such as relatives of CD patients are easily identifiable and are known to be at heightened risk of developing the disease. We want to identify individuals who are at the greatest risk of CD and will benefit the most from early intervention. We will look at known genetic risk factors for CD to compile genome wide polygenic risk scores and use a combination of immune biomarkers and images of the bowel such as those captured by a Pill Camera to identify early signs of intestinal inflammation in asymptomatic individuals. We will then employ machine-learning to analyse the data. These methods can look for patterns in data and learn from them so that future outcomes (such as whether an individual will develop CD) can be predicted. We have previously tested this with just a limited number of genetic markers, with great success (Taylor et al, 2020). Our goal is to build on these methods by incorporating whole-genome data and data collected from a 10-year follow-up of these relatives and perform validation in population-based datasets (UK Biobank and NIHR IBD Bioresources). This is a highly novel and untried approach to early detection in CD. We proposed that our predictive test could be implemented in the diagnosis pathway for CD by selectively targeting high risk groups and identifying those who are most likely to develop disease for closer monitoring and early therapy.

Project 45 - Effects of sex chromosome complement during early human embryogenesis

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Abstract

Aneuploidy (aberrations in chromosome number) is the most commonly identified chromosome abnormality in humans, occurring in at least 5% of all clinically recognized pregnancies. Incidence is even greater in pre-implantation embryos at around 20%. In humans, sex chromosome aneuploidies (SCAs) form the largest class of chromosome abnormalities – i.e. Turner's syndrome (XO), Klinefelter syndrome (XXY), XYY and Trisomy X. The effects of sex chromosome complement during early development remains incompletely described. Unlike autosomal aneuploidy which typically have deleterious consequences, SCAs are frequently associated with live births. Individuals with SCA often require life-long clinical input, ranging from multi-system problems experienced in cardiovascular, cerebral, endocrine, and gonadal function. Thus, understanding the pathophysiology of how these issues arise during development remains an important area of research. We hypothesise that global investigation of early human embryo and placenta development may allow for the detection of primary alterations underlying these pathophysiologies. For example, early trophoblast development plays a key role in determining the final size and shape of the placenta, and so may later impact development of the foetal heart. The host labs will investigate these effects using a combination of cutting-edge techniques, including next-generation sequencing, microscopy, and advanced embryo culture systems.

Surplus SCA embryos will be identified following pre-implantation genetic diagnosis during in vitro fertilisation (IVF) treatment and used for this research. Sex chromosome euploid embryos will be used as controls. We will apply techniques that allow contemporaneous interrogation of the genome and transcriptome to derive high-quality multimodal datasets. Genomic profiling will include assaying chromatin accessibility (ATAC-seq) and DNA methylation (e.g. RRBS). Other embryos will be subjected to 3D microscopy to assay for lineage specification and early placenta development. Host lab expertise in human embryo manipulation, disaggregation, microscopy, and advanced genomics techniques will provide training to the doctoral candidate. Further exposure to computational biology will also be expected.

The research will be of broad interest to the scientific community of developmental biologists, and also inform clinicians and patients alike on how aneuploidy and sex chromosome functions might affect the emergence of deleterious phenotypes later in life.

Year 1: optimisation of -omics approaches (e.g. RNA, ATAC-seq and RRBS techniques) in ES-cells / trophoblast stem cells / organoids to recapitulate cell number of embryo.
Year 2: generation of control embryo and SCA embryo datasets. Commence validation of extended culture model and characterisation of trophoblast and embryonic lineages by confocal imaging.
Courses

Year 3: Data analysis and validation of candidates in embryos by immunofluorescence microscopy.
Year 4: Completion of critical experiments; write-up findings - manuscript and thesis.