UNIVERSITY OF BIRMINGHAM

College of Medical and Dental Sciences

Intercalated BMedSc Clinical Sciences

Research projects 2013/14
On your application form, you need to state your preferences for up to four research projects from this booklet. You are expected to consult the relevant supervisor before applying.

Application forms are available online: [http://www.birmingham.ac.uk/students/courses/undergraduate/med/ClinicalScienceBMedSc-IntercalatedDegree.aspx](http://www.birmingham.ac.uk/students/courses/undergraduate/med/ClinicalScienceBMedSc-IntercalatedDegree.aspx) or please contact the course administrator, Miss Yvonne Palmer in the Division of Cancer Studies: [Y.Palmer@bham.ac.uk](mailto:Y.Palmer@bham.ac.uk) Telephone: 0121 414 8099.

**Ethical considerations**

We understand that students' ethical standards and convictions vary and may influence the type of work you feel you can conduct throughout the course. Students should be assured that all projects have ethics approval if required. However it is possible that personal convictions may be brought into conflict with the nature of the work you are conducting. For example some projects involve the use of cell lines that have been derived from human embryonic tissue. Other products involve use of animal matter and animal testing. Please talk directly to your supervisor from the outset about the exact nature of the materials, methods and procedures in the projects you are interested in.
Lead Supervisor: Dr Patricia Lalor

Co Supervisor: Dr Ye Oo and Dr Josephine Bunch

Project Title: Development of next-generational imaging technology for candidate biomarker identification in Primary Sclerosing Cholangitis

Department: Centre for Liver Research, Immunity and Infection

Contact Email: p.f.lalor@bham.ac.uk

Is the project cancer related? Yes or No

Discipline:
- Cancer Sciences
- Pathology
- Metabolic Medicine
- Haematology
- Infection
- Immunology
- Anatomy
- Endocrinology
- Liver & GI Medicine
- Evolutionary biology in Clinical medicine

Project Outline
Primary Sclerosing cholangitis (PSC) is a chronic inflammatory liver disease linked to damage of intrahepatic bile ducts which leads to liver damage, carcinogenesis and cirrhosis. There are currently no treatment options available other than liver transplantation and no good serological biomarkers which can be used to accurately diagnose the condition. Similarly although likely autoimmune in nature, the cause of the disease remains to be identified. Occurrence of PSC is closely linked to ulcerative colitis, there is a male predominance and a prevalence of 22–8.5 per 100,000. Up to 15% of patients will go on to develop hepatic cancer and the median time from diagnosis until liver transplantation or death is ten years with a life expectancy of 25 years. Thus there is an urgent need to identify biomarkers that can aid differential diagnosis of PSC, be used to assess/predict progression and shed some light onto disease pathogenesis. We have recently developed MALDI-imaging based technology which uses a non-targetted multivariate approach combined with principal component analysis to identify profiles of biological molecules which can be used to distinguish liver specimen types (Palmer et al under review). We wish to repeat this analysis using PSC liver and serum specimens to identify candidate biomarkers, assess whether they are present in serum and to localise their expression within tissue. This project will therefore begin to assess the potential of MALDI-imaging for candidate biomarker identification in human liver disease and is of importance for a particularly challenging condition. The project will be of relevance for students with an interest in Liver and GI medicine, Inflammation, Cell Biology, Biochemistry, Next generational imaging approaches, Hepatology and Molecular Biology.

Techniques to be used in the project: MALDI-imaging, immunocytochemistry, qPCR, human cell culture, ELISA, functional assays

**How are you planning to ensure adequate supervision?**

We have chosen to combine the technical expertise of clinical and academic supervisors to ensure the student is exposed to the maximal number of transferable research skills. All supervisors have a proven track record in supervision of both undergraduate and postgraduate students (clinical and scientific) and work in large research groups providing the student with exposure to research staff at all stages of their research careers. Appropriate training in research techniques and data analysis and presentation will be supplied. We will have weekly meetings with supervisors and interactions with other members of the research groups, who will be available day to day for advice and tuition.

**The student role.**

The student will be expected to perform experiments and conduct data analysis. They will present data to the supervisory team and wider research groups and be expected to assimilate available published literature under guidance from supervisors. They will be trained and expected to work using good laboratory practice under local standard operating procedures and be mindful of ethical use of human tissue specimens. They will be working in large research teams and thus would be expected to interact with colleagues and use local facilities in a respectful manner.
Lead Supervisor: Dr Patricia Lalor

Co Supervisor: Dr Lia Liasko

Project Title: Expression and function of APOER2 in human fatty liver disease.

Department: Centre for Liver Research, Immunity and Infection

Contact Email: p.f.lalor@bham.ac.uk
Telephone: x46967

Is the project cancer related? Yes or No

Discipline: Cancer Sciences Immunology
Pathology Anatomy
Metabolic Medicine Endocrinology
Haematology Liver & GI Medicine
Infection Evolutionary biology in Clinical medicine

Project Outline
Liver disease is the 5th most common cause of death in the UK and although alcohol is the chief cause, damage resulting from fatty liver disease is similarly increasing. Apolipoprotein R receptor 2 (APOER2/LRP8) is a member of the low density lipoprotein receptor (LDLR) family with roles in both signal transduction, receptor mediated endocytosis and as a receptor for the cholesterol transport protein apolipoprotein E. Expression of this gene is a marker for major depressive disorder, and has been linked to the pathogenesis of Alzheimer’s disease. We have recently demonstrated hepatic upregulation of the receptor in fatty liver disease and have demonstrated that hepatic factors characterizing metabolic syndrome can upregulate expression at mRNA and protein level. Similar factors have been linked to the pathogenesis of Alzheimer’s disease. We now wish to characterize on a cellular level regulation of expression of APOER2 on human liver cell populations, and to determine whether upregulation of expression has functional consequences in fatty liver disease. We hope to elucidate whether common mechanisms may drive upregulation of APOER2 at distinct anatomical sites leading to disease pathology.

Techniques to be used in the project: human cell culture and isolation, confocal microscopy, qPCR, immunocytochemistry, functional assays (lipid uptake, RNAi, ligand binding assays)

### How are you planning to ensure adequate supervision?

We have chosen to combine the technical expertise of two supervisors to ensure the student is exposed to the maximal number of transferable research skills. All supervisors have a proven track record in supervision of both undergraduate and postgraduate students and work in a large research group providing the student with exposure to research staff at all stages of their research careers. Appropriate training in research techniques and data analysis and presentation will be supplied. We will have weekly meetings with supervisors and interactions with other members of the research groups, who will be available day to day for advice and tuition.

### The student role.

The student will be expected to perform experiments and conduct data analysis. They will present data to the supervisory team and wider research groups and be expected to assimilate available published literature under guidance from supervisors. They will be trained and expected to work using good laboratory practice under local standard operating procedures and be mindful of ethical use of human tissue specimens. They will be working in large research teams and thus would be expected to interact with colleagues and use local facilities in a respectful manner.
### Lead Supervisor:
Dr Naeem Khan

### Co Supervisor
Dr Sylvie Freeman

### Project Title:
Assessing mitochondrial readiness for cell death in myeloid malignancies: a potential biomarker for predicting treatment outcome

### Department:
Clinical Immunology

### Contact Email:
Telephone: n.khan.2@bham.ac.uk

### Is the project cancer related?
Yes

### Discipline:

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<th>Cancer Sciences</th>
<th>Immunology</th>
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### Project Outline

Acute myeloid leukaemia (AML) and high-risk myelodysplastic syndromes (MDS) are aggressive stem-cell disorders for which cytotoxic chemotherapy is current first-line treatment. Initial responses to chemotherapy are variable with some patients responding well and staying disease-free, while others respond briefly or do not respond at all. Predicting and understanding the response to chemotherapy is thus crucial for improved patient outcome.

Recent evidence [ref.1] suggests that the different outcomes after chemotherapy depend on the intracellular mitochondrial readiness for cell death (apoptosis). This is affected by the balance between pro-apoptotic and anti-apoptotic BCL-2 proteins. Cells with more pro-apoptotic/less anti-apoptotic profiles are highly primed for death and very sensitive to chemotherapy drugs. Conversely, less-primed cells are able to resist drug-induced cell death.

This project aims to test if the pre-treatment mitochondrial status of patient cells would be an improved predictor of responses to chemotherapy in AML and high-risk MDS. The level of priming will be determined using an assay which measures mitochondrial integrity by immunofluorescence [ref.2].

The student selecting this project will work in a busy clinical research laboratory where we have direct access and ethical approval to study samples from a large group of adult AML and MDS patients currently enrolled on national clinical trials. Techniques will include cell isolation from patient bone marrow and blood, in vitro cell culture, drug chemosensitivity assays and flow cytometry. The data from this study would have high potential for publication and be of great interest to local and national clinicians aiming to improve treatment of AML/MDS patients under their care.

### References


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<td>I will accompany the student during the initial stages of the research project and provide direct training in each of the experimental techniques. Training will also be provided in literature searches and data analysis. I will also meet briefly with the student on a day-to-day basis for brief discussions and hold more detailed once-weekly meetings to monitor overall progress and future experiments. The co-supervisor Dr Freeman will provide feedback and ideas in clinical and scientific matters in meetings held every 4-6 weeks.</td>
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<td>The student will be expected to drive the project forward by carrying out laboratory experiments and engaging the literature. With training, the student will also be expected subsequently to develop expertise in data analysis and carry out oral presentations to the research group(s) within the department. At the end of the project, the student will submit a research thesis in the College-recommended format.</td>
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Project Outline

Exaggerated fibrosis is a feature of many chronic disease especially in the lung, kidney and liver,. In lung disease, inflammatory alveolitis leads to pulmonary fibrosis, but in other diseases such as idiopathic pulmonary fibrosis, inflammation is not a marked feature. In this situation it is believed that aberrant wound repair processes involving epithelial and fibroblast cross-talk promotes lung damage, a process that may involve epithelial mesenchymal transformation (EMT). This is important since idiopathic pulmonary fibrosis (IPF) is a devastating disease with an average life expectancy from diagnosis of 2.5 years with five year survival of between 20 and 40%. Currently there are no therapies proven to reduce mortality and only one drug, pirfenidone, is licensed for use in Europe that may slow the progression of the disease. Clearly developing novel targets for therapy for pulmonary fibrosis patients is a major unmet health need.

Recent work by members of the Centre for Translational Inflammation Research (CITR) have identified a role for the mesenchymal cell marker CD248, also known as endosialin, in the pathogenesis of kidney fibrosis. CD248 expression is prognostically important in human chronic kidney disease and CD248 knock out mice are protected against experimental fibrosis of the kidney. CD248 appears to regulate signalling via the PDGF receptor There is no published information on CD248 expression in human lung disease other than lung cancer.

The aims of this project are therefore to:
1. Establish using immunohistochemistry / fluorescence microscopy the expression of CD248 and other stromal markers in surgical lung biopsies and lung transplant explants.
2. To relate expression of the stromal to markers of clinical severity – e.g. lung function.
3. To use normal primary lung epithelial cells (alveolar type II and fibroblasts) to look at regulation of CD248 expression by cytokines and growth factors such as TGF-beta and IL-4 / IL-13 as well as hypoxia.
## How are you planning to ensure adequate supervision?

Both the supervisors are located in the new Centre for Translational Inflammation Research in the University Labs in the new QEHB. Weekly supervisor meetings will be arranged to ensure student progress and to plan the evolution of the project. The student will attend both rheumatology and respiratory research in progress meetings weekly.

Considerable expertise exists within the rheumatology and respiratory research groups in the techniques necessary to make this project successful. A large number of patient samples are available for the tissue work in collaboration with Mr Babu Naidu, thoracic surgeon, and Dr Gerald Langman, consultant thoracic histopathologist at Heartlands. Regular updates of progress with these collaborators will ensure detailed multidisciplinary support for the successful student.

Day to day support of the student in the laboratory work will be provided by post-doctoral researchers (Dr Adam Croft, Dr Stuart Smith, Dr Dom Bartis and Dr Sian Lax) who have expertise in the immunohistochemistry, CD248 biology, lung fibroblast and epithelial cell extraction, flow cytometry and necessary cell culture techniques.

## The student role.

The student will perform the tissue immunohistochemistry and fluorescence microscopy. The student will score the level of expression of CD248 in the lung tissue and gather clinical data on the patients from available outcome database.

The student will learn cell culture techniques and the basics of primary cell extraction from lung resection specimens. The student will perform stimulation experiments to look at CD248 expression upon lung derived fibroblasts and epithelial cells that have undergone EMT.
Primary CNS lymphoma (PCNSL) is defined as a lymphoma confined to the central nervous system. Compared with extra-cerebral lymphomas, PCNSL has a poorer prognosis; 5-year survival rates are less than 20%. Intensified therapy and the use of multiple agents may improve survival, but are associated with significant toxicity. An improved understanding of the pathogenesis of PCNSL is required for the development of novel therapeutic approaches.

The risk of PCNSL is increased in individuals with acquired (e.g. AIDS), iatrogenic (e.g. recipients of organ transplants) or congenital immunodeficiency. The Epstein-Barr virus (EBV), an oncogenic herpesvirus, is detectable in the tumour cells of the majority of PCNSL arising in immunosuppressed individuals and is believed to be the primary causative agent in this disease. CNS lymphomas arising in immunocompetent individuals are usually EBV-negative and probably represent a biologically distinct entity.

Attempts to understand the underlying biology of PCNSL including is intrinsic heterogeneity and the extent to which it relates to systemic diffuse large B cell lymphomas have been frustrated by several problems. Prominent among these are technical difficulties which result in the contamination of the lymphoma cells from surrounding CNS tissue during their isolation and analysis. Furthermore, there are no representative cell line models.

The overarching aim of this project is to develop better tools to study PCNSL and to use these to explore the pathogenesis of this poorly understood condition. In doing so we hope to be able to uncover the key cellular pathways leading to this disease that could ultimately be used as targets for novel therapies.

We will use techniques pioneered in our laboratory and based on magnetic bead technology to isolate and purify for the first time primary lymphoma cells from patients with this disease. Fresh PCNSL are already being routinely collected as part of the University of Birmingham Brain Tumour Collection, where up to 25 patients are seen through MDT activity per annum. We will analyse these cells in terms of...
proliferation and apoptosis sensitivity, and perform gene expression studies. In parallel we will describe their immunophenotype using standard methods available in our laboratory.

### How are you planning to ensure adequate supervision?

The student will be part of a large group which is already studying the pathogenesis of B cell lymphomas and which receives substantial grant support from *Leukaemia Lymphoma Research*. The student will receive on the bench supervision from post-doctoral fellows with substantial experience of the isolation and analysis of B cell subsets from primary tissues. The student will also benefit from the expertise of Dr Detta who has many years experience of the culture of brain cancer cells.

### The student role.

The student is expected to be highly motivated and prepared to work out of hours when necessary. The student will also be expected to liaise with surgeons and other clinicians to collect samples and analyse patient data.
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<th>Lead Supervisor:</th>
<th>Dr. Frederick Chen, Consultant Haematologist</th>
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<tr>
<td>Co Supervisor</td>
<td>Dr. Rasoul Amel-Kashipaz, Consultant Histopathologist</td>
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<tr>
<td>Project Title:</td>
<td>Analysing the protein expression of a novel cancer antigen in myeloma cells from histological material</td>
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<tr>
<td>Department:</td>
<td>Depts. of Haematology and Histopathology</td>
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<tr>
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**Project Outline**

Cancer cells express proteins that are not expressed by normal cells. These proteins can be used as markers of disease and as targets of treatment. Cancer Testis antigens belong to this category of proteins. They are expressed during embryogenesis but downregulated after birth except for the testis which is an immunologically privileged site. Published molecular studies suggest that the cancer antigen HAGE is expressed in myeloma cells (1). This study attempts to find out whether the actual protein encoded by the gene is also expressed in myeloma specimens and to what extent, and whether the antigen can be used as an anti-myeloma therapeutic target and biomarker.

HAGE is a Cancer Testis Antigen found to be expressed in myeloma cells at the RNA level by microarray studies (1, 2) but not in normal tissues. It has also been shown to be expressed in approximately 10% of patients with carcinoma of the breast and associated with a significantly worse prognosis in a series of 1400 patients (unpublished data from Nottingham). Published work on CML indicated that the expression of this gene is associated with advanced chronic phase CML in the process of transformation (3, 4). HAGE is a helicase antigen involved in cell cycle and RNA metabolism and there is evidence that the protein is associated with cellular proliferation and is potentially a marker of advanced disease (5).

The objectives of the study are:

1. to validate immunocytochemistry staining of novel anti-HAGE antibody
2. to assess the expression of the cancer antigen in myeloma histology specimens
3. To correlate the histological findings with clinical outcome data

We have already validated the staining techniques using the novel antibody to HAGE (DDX43) on formalin fixed histological tissue and on decalcified bone marrow trephines from myeloma patients.

The first batch of staining of myeloma specimens have demonstrated for the 1st time the expression of the HAGE protein.

We have so far examined positive (n=2) and negative control specimens (n=3). We
have stained and analysed diagnostic histology specimens from a variety of haematological malignancies (high grade B-cell lymphoma, Burkitt’s lymphoma, Marginal zone lymphoma, lymphoplasmacytic lymphoma, B CLL, Acute Myeloid Leukaemia, plamablasic lymphoma and found no protein expression.

In 7 myeloma biopsies, we have found 4 to be positive for either nuclear or cytoplasmic staining (57%) (see figure). The other haematological malignancies did not stain positively, although to date the numbers examined are small.

We plan to analyse up to 50-60 myeloma histology specimens to obtain a statistically significant result and thereafter to study the T cell response to newly identified HLA-restricted HAGE peptides in peripheral blood. The histological staining is in progress in the routine lab.

This work is ethically approved

References

(1) M Condomines et al. JI, 178; 2007:3307;
(2) Martelange V et al., Cancer Res 2000; 60: 3848
(3) Roman-Gomez J, Haematologica 2007; 92: 153
(4) Adams SP et al., Leukemia 2002; 16: 2238
(5) Morgan G. Mathieu et al., Cancer Immunity 2010, 10;2

The student role.

The student joining the project will be able to learn about immunocytochemistry and be involved in the microscopy of the stained myeloma specimens. He/she will evaluate the diagnosis and staining results of the slides under the supervision of the consultant histopathologist and correlate the histological findings with patient outcome data in the department of haematology. During this attachment the student will be able to learn the basic skills of clinical histopathological diagnosis and acquire an insight into the histology of haematological malignancies, which is regarded as one of the more challenging areas of histopathology. The student will also be involved in data analysis and use statistical methods. Depending on time the student may be able to do some cellular immunology assays. As positively staining specimens have already been identified, it is likely that this work will be informative and reportable in a peer-reviewed journal.
Proper genome maintenance is crucial for normal development and prevention of diseases such as cancer. In eukaryotic cells, genomic DNA is packaged into chromatin: a highly architectural DNA-histone complex. DNA strand is wrapped around nucleosomes formed of eight histones (2xH2A, 2xH2B, H3 and H4). The histone tails protrude from the nucleosome and are subjected to an array of post-translational modifications (phosphorylation, acetylation, ubiquitylation, methylation), which work together to regulate chromatin structure.

Chromatin organization has profound influence on DNA-templated processes such as transcription, DNA replication and DNA repair. Not surprisingly, many histones modifying enzymes have been identified as oncogene or tumor suppressors. Importantly, the number of connections between histone ubiquitylation and cancer development is increasing every day.

We have observed recently in our studies a novel ubiquitylation of histone H3 during DNA replication. The aim of the proposed project is therefore to study this modification in detail: to identify the timing, requirements and enzymes inflicting this modification and to attempt unravelling its importance for cancer development. The Student will also examine potential DNA replication dependant ubiquitylation of other histones.

To conduct this research, the Student will use a cell-free system that recapitulates a whole round of DNA replication in vitro and thus is invaluable for biochemical studies of eukaryotic DNA replication and DNA repair processes.

**Further reading:**
Hoeller D, Dikic I. Targeting the ubiquitin system in cancer therapy.
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<td>I will supervise the Student myself on a day to day basis and teach the student all the techniques required. I will make sure that the Student understands the project in depth, that her / his lab book is kept up to date and that we discuss the progress of the project on the regular basis. Finally, I plan to ask the student to prepare a number of presentations about different aspects of the project to ensure that he / she gathers all required literature background knowledge over the duration of the project rather than leaving it till the end.</td>
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<td>I expect the Student to become proficient in all the techniques he / she will need to use and to be able to carry on the experiments by him / herself after the initial training. All of the work carried out by the Student for the purpose of this project is laboratory based (wet science). All of the research conducted by the Student will be novel and hopefully will result in creating data that will be used for future grant applications and publications. The Student will be a co-author of any publication resulting from this project.</td>
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Non-alcoholic fatty liver disease (NAFLD) is tightly associated with obesity and type 2 diabetes and is the fastest growing cause of severe liver disease in the western world. It is a spectrum of disease that can ultimately lead to cirrhosis and the need for transplantation. Additionally, NAFLD patients have an increased risk of developing hepatocellular carcinoma (HCC). However the biological processes that underpin its pathogenesis and the progression to cirrhosis and cancer are not clearly defined. Our hypothesis is that androgen exposure to the liver may be crucial in regulating the amount of lipid that can accumulate within hepatocytes and that androgens can, directly or indirectly, drive tumour initiation. In addition, we believe that the expression of enzymes that metabolise androgens within the liver have the potential to offer a novel therapeutic target.

As part of this project, the expression of steroid hormone receptors and androgen metabolizing enzymes will be characterized in human hepatoma cell lines and primary hepatocytes cultures. We will explore the impact of differing times and concentrations of androgen exposure to proliferation, apoptosis and the accumulation and utilisation of lipid in hepatocytes. We will also investigate whether pharmacological and genetic manipulation of androgen metabolising enzyme activity impacts upon these readouts. Additionally, in liver samples taken from mice treated with androgens we will measure the impact of treatment on histological and cancer markers.

How are you planning to ensure adequate supervision?

The student will join a group of clinical and basic scientists headed by Dr Jeremy Tomlinson, a consultant endocrinologist and MRC senior clinical fellow. In the lab they will be supervised directly by a post doctoral researcher and 3rd year PhD student. Additionally, there are several clinicians and scientists within the wider group that have specialist expertise and knowledge.
The student role.

The student will learn a broad range of molecular techniques, using a combination of *in vitro* (generated by the student) and *in vivo* samples (from a bank of samples). All techniques are established in the lab.

Cell culture (human hepatoma cell lines and primary human hepatocyte cultures).
RNA extraction and reverse transcription
Conventional and real time PCR
Dynamic isotope assays of lipid flux (lipogenesis, β-oxidation and fatty acid uptake)
Western blotting (total protein and phospho-protein)
Apoptosis assays
Proliferation assays
Histological scoring
immunohistochemistry

In combination with other data it is hoped that this work will contribute to a high impact publication and that the student will be involved in the data analysis and presentation.

In addition to bench work the student will attend and present at weekly lab meetings.
Lead Supervisor: Dr Parth Narendran

Co Supervisor: Prof. Ed Rainger

Project Title: Quantification of a novel anti-inflammatory pathway using mass spectrometry

Department: School of Clinical and Experimental Medicine

Contact Email: p.narendran@bham.ac.uk

Telephone: 

Is the project cancer related? No

Discipline: Cancer Sciences Immunology
Pathology
Metabolic Medicine Anatomy
Haematology Endocrinology
Infection Liver & GI Medicine
Evolutionary biology in Clinical medicine

Project Outline

We recently identified a novel pathway that regulates the recruitment of T-lymphocytes across inflamed endothelium. This anti-inflammatory pathway involves adiponectin, an anti-inflammatory cytokine produced by the adipose tissue [1], known to modulate leukocyte recruitment in vivo [2]. In our laboratory, we have shown that adiponectin inhibits T-lymphocyte migration across inflamed endothelium. Adiponectin does not directly target T-lymphocytes; rather it stimulates B-lymphocytes to secrete a novel endogenous peptide (PEPITEM) that in turn modulates T-lymphocyte recruitment (Figure 1). This novel regulatory peptide stimulates the release of sphingosine-1-phosphate (S1P) by the endothelium that in turn modulates T-lymphocyte recruitment. S1P is a sphingophospholipid known to regulate T cell egress from the secondary lymphoid organs by interaction with its receptors 1 and 4 expressed on T cells (S1PR1/4). However, its role in the periphery is not well characterised [3, 4]. Interestingly, the adiponectin/PEPITEM pathway is altered in autoimmune and chronic inflammatory diseases such as type 1 diabetes, and is therefore an exciting potential therapeutic target.

We hypothesize that levels of PEPITEM differ in patients with autoimmune and chronic inflammatory diseases. Our overall aim is to accurately quantify this pathway using recently developed protocols from our laboratory.
Figure 1: Schematic representation of the endogenous B-cell mediated regulation of T-cell migration during inflammation. Adiponectin induces PEPITEM secretion from B-cells. PEPITEM signalling through an unknown receptor on the endothelium results in S1P production, which subsequently inhibits T-cell migration. (AR1/2: adiponectin receptors 1 and 2; S1P: sphingosine-1-phosphate; S1P1/4: S1P receptors 1 and 4).

Plan of investigation:

Aim 1: Quantification of PEPITEM secretion by B cells in health and type 1 diabetes

Adiponectin loses its capacity to inhibit the transmigration of T cells across inflamed endothelium in patients with type 1 diabetes compared to healthy controls. The loss of control on T cell recruitment correlates with the level of expression of adiponectin receptors 1 and 2 (AR1/2) on B cells. However, the amount of PEPITEM that can be released by B cells under adiponectin stimulation is still unknown. Our hypothesis is that B cells from patients with type 1 diabetes lose their capacity to produce sufficient PEPITEM. Here, we would like to quantify PEPITEM release by B cells using a stable tritium-labelled PEPITEM as an internal standard. B cells will be isolated from healthy controls, patients with type 1 (T1D) or type 2 diabetes (T2D), and stimulated with adiponectin following our well established protocol. B cell supernatants will be spiked with a known amount of the tritium-labelled PEPITEM which will allow quantification by liquid chromatography coupled to mass spectrometry (LC-MS/MS). In addition, we would like to identify which B cell subset is responsible for PEPITEM release. Using commercially available kits, we propose to select for the different B cell subsets and measure their capacity to produce PEPITEM.

Aim 2: Characterisation of sphingosine-1-phosphate production by endothelial cells

Our data demonstrate the capacity PEPITEM to induce S1P release by endothelial cells. We have used S1P receptors antagonists and sphingosine kinases inhibitors to show the involvement of S1P in the adiponectin/PEPITEM pathway. However, we would like to accurately quantify the amount of S1P generated by the endothelium under PEPITEM stimulation. Supernatents of endothelial cells stimulated with PEPITEM will be collected and lipids will be extracted using methanol/chloroform extraction. Using a known quantity of S1P standard, we will quantify S1P in these samples using LC-MS/MS.

Aim 3: Measurement of circulating PEPITEM in diabetes

We have shown that adiponectin induces B-lymphocytes to secrete PEPITEM in vitro. Thus, circulating B-lymphocytes are likely to release detectable amounts of PEPITEM into the plasma in response to adiponectin, which is present at high concentrations in the circulation. We believe that low adiponectin receptor expression
on B-lymphocytes is associated with lower levels of circulating PEPITEM. Therefore, our aim is to quantify the circulating levels of PEPITEM and correlate these with the expression of adiponectin receptors on B-lymphocytes, in healthy controls, T1D and T2D. Levels of plasma B-cell-derived PEPITEM will be measured using LC-MS/MS. Samples will be spiked with a known amount of the tritium-labelled PEPITEM, purified and quantified using the relative intensities of the labelled and unlabelled peptide ions. Expression of adiponectin receptors on B-lymphocytes will be quantified by flow cytometry and real-time PCR using well-established protocols in our laboratory.

References
3. Pham T et al. (2008) S1P1 receptor signalling overrides retention mediated by G alpha i-coupled receptors to promote T cell egress. Immunity. 28(1):122-123

How are you planning to ensure adequate supervision?
The student will join the well-established collaborative research group of Dr Narendran and Prof. Rainger within the Institute for Biomedical Research. This involves meeting on a weekly basis with the core of people working on the PEPITEM project. These meetings will allow informal and supportive discussion of the student’s progress and establishment of short and long term plans. The student will be supervised in the laboratory on a daily basis by an experienced post doctoral fellow who conducted her PhD on PEPITEM (Dr Myriam Chimen).

The student role.
This project will address some remaining questions of the mechanism by which adiponectin regulates T-lymphocyte migration across inflamed endothelium. The student will learn a variety of useful and cutting-edge techniques including immune cell isolation and culture, culture of endothelial cells, flow cytometry, real-time PCR, protein and lipid biochemistry and mass spectrometry in collaboration with Dr Ashley Martin (Cancer sciences). The student will be involved in in-vitro work with human tissue only. The student will be required to read around the subject, present and participate in lab meetings and will be in a supportive laboratory atmosphere. The student will be responsible for analysing the data and keep detailed record of the work accomplished. Upon initial training, the student will be able to gain independence in the lab, contribute to experimental design and present the data in an appropriate manner. The student will have many opportunities to interact with lab members who will kindly offer support at a daily basis.
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<tr>
<th><strong>Primary Supervisor:</strong></th>
<th>Padma-Sheela Jayaraman</th>
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<tr>
<td><strong>Project Title:</strong></td>
<td>Hypoxia and the Regulation of tumour cell growth/migration by the PRH transcription factor</td>
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<tr>
<td><strong>Department:</strong></td>
<td>School of Immunity and Infection</td>
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<tr>
<td><strong>Contact Details:</strong></td>
<td><a href="mailto:p.jayaraman@bham.ac.uk">p.jayaraman@bham.ac.uk</a></td>
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| **Is the project cancer related?** | Yes |

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<td>Evolutionary basis of clinical medicine</td>
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**Project Outline**

We are interested in the regulatory pathways that control the proliferation of normal mammary epithelial cells and the events that disrupt this control in tumourigenesis. PRH /Hhex (Proline Rich Homeodomain) protein is an important regulator of haematopoiesis, vasculogenesis and formation of many organs. In the adult the misregulation of PRH is associated with leukaemias, and breast and thyroid cancers. The VEGF signalling pathway is commonly disregulated in cancer and increased signalling is associated with increased cell survival and cell proliferation. My group has shown that PRH is a transcriptional repressor of multiple genes in the VEGF signalling pathway and that the regulation of VEGF signalling by PRH controls leukaemic growth. We have also shown that treatments for leukaemic growth influence PRH activity and in the absence of PRH the treatments are significantly less effective so PRH is a potential therapeutic target.

Our current research into breast cell proliferation has revealed that PRH inhibits the growth and the migration of normal and breast tumour cells. We have also identified some of the target genes involved in regulation by PRH in breast cells. We know that PRH is down regulated at the mRNA level in breast tumour cells under hypoxia. We are interested to determine whether PRH (i) growth inhibition activity (ii) migration activity is down regulated under hypoxia.

To investigate this we will examine PRH protein levels, PRH subcellular localisation, PRH DNA binding, and transcription activity under hypoxia and normoxia. Cell types may include breast normal and tumour cells. Prostate normal and tumour cells. Thyroid tumour cells.

Techniques include cell culture, transient transfections, Western blotting, cell fractionation, immunostaining, chromatin Immunoprecipitation, RT-PCR. transwell migration assays.

**Refs:** Noy et al., 2010 Mol. Cell Biol. 30;2120

Noy et al., 2012 Leukaemia Research 36;1434
A postdoctoral research fellow investigating the regulation of tumour cell migration by PRH will be on hand to offer daily supervision in the laboratory. Dr Jayaraman is available for individual weekly meetings and to offer guidance on the overall strategy of the project and in addition weekly lab meetings are held. In addition inter-laboratory meetings with principal collaborators are held periodically and the student is expected to attend these meetings.

The student role.

The student has to be dedicated to eight months of practical work in the laboratory and must provide enthusiasm and meticulous planning for the execution of repeatable and well controlled experiments. The project is a basic research project that will yield novel results in understanding the control of the growth and migration of tumours. Reading around the subject and good time management are essential.
Platelets are small anucleate blood cells that form thrombi in damaged blood vessels and prevent excessive bleeding following injury. They respond rapidly to vascular injury, but remain in a resting state under normal conditions. Activation receptors on the platelet surface transmit signals within platelets that cause them to undergo dramatic morphological and biochemical changes and become prothrombotic. Understanding the molecular basis of how platelets are activated and mediate thrombosis has important clinical implications for the prevention of deep vein thrombosis, embolization, myocardial infarction and stroke, which combined are the leading causes of death in the western world.

Src family kinases (SFKs) are a family of enzymes that initiate and propagate signals from a variety of platelet surface receptors, including the immunoreceptor tyrosine-based activation motif (ITAM)-containing collagen receptor complex GPVI-FcR γ-chain and the fibrinogen integrin αIIbβ3. The primary objective of this project will be to determine how the kinase-phosphatase pair C-terminal Src kinase (Csk)-CD148 regulates SFKs and the thrombotic potential of platelets. Pharmacological inhibition and genetic deletion of SFKs in mice attenuates platelet activation and thrombosis. However, remains unclear how SFKs are regulated in platelets. We recently demonstrated that the receptor-like protein-tyrosine phosphatase (PTP) CD148 is a critical positive regulator of SFKs in platelets (Senis et. al. Blood 2009; Ellison et. al. JTH 2010; Mori et. al. ATVB 2012). It does so by dephosphorylated an inhibitory tyrosine in the C-terminal tail of all SFKs. However, little is known about how SFKs are inactivated in platelets. The main inhibitor of SFKs in other cell types is Csk, which phosphorylates the C-terminal inhibitory tyrosine of SFKs and maintains them in an inactive state. Based on this evidence, we hypothesize that Csk works in conjunction with CD148 to maintain an optimal level of SFK activity in platelets. This allows platelets to respond rapidly to vascular injury without becoming pre-activated and forming life-threatening thrombi.

Hypothesis
The kinase-phosphatase pair Csk-CD148 is a critical molecular switch that regulates an optimal level of SFK activity in platelets and their reactivity to vascular injury. Csk and CD148 single and double conditional knockout (KO) mouse models will be used
to test our hypothesis.

**Objectives**

1) To determine the effects of deleting Csk and CD148 on platelet function.
2) To determine the effects of deleting Csk and CD148 on signalling from collagen receptor complex GPVI-FcR γ-chain and the fibrinogen integrin αIIbβ3.
3) To determine the effect of deleting Csk and CD148 on thrombus formation in mice

**How are you planning to ensure adequate supervision?**

The student will be jointly supervised by Drs Mori and Senis. Dr Mori is a senior postdoc in the Senis group who has supervised numerous undergraduate and postgraduate students in the Birmingham Platelet Group over the past five years. She has experience with all of the assays the student will be using and will be working closely with the student on a day-to-day basis. Dr Mori is the named postdoc on a BHF funded project grant on the same area of investigation, thus working together will be mutually beneficial.

Dr Senis has supervised numerous undergraduate and graduate students and postdocs over the past ten years. Dr Senis will be meeting with the student and Jun on a weekly basis to design experiments, interpret data and plan future experiments. Dr Senis will also assist with training the student in the lab, and provide guidance in writing reports and preparing oral presentations for lab meetings and as part of the course.

Dr Senis shares lab space with Professor Steve Watson and Drs Steve Thomas and Neil Morgan. Thus, the student will be surrounded by other students and postdocs, who will also provide guidance with general lab practice, equipment use and scholarly discussion. This is an excellent academic environment for a student to train in.

**The student role.**

*Csk* and *CD148* conditional KO mouse models have already been established in the Senis group, by Dr. Mori. Although the *Csk* conditional KO mutation appears to be lethal, the student will be able to study platelets from *Csk* heterozygous deficient mice and *CD148* conditional KO mice. Dr Mori is in the process of generating a Tamoxifen-inducible *Csk* KO mouse model to circumvent the issue of the apparent lethality of the *PF4-Cre*-mediated *Csk* conditional KO.

The student will analyse platelets from *Csk* and *CD148* conditional KO mouse models for functional and biochemical defects. Classical platelet functional and biochemical assays will be employed that are well established in the Birmingham Platelet Group and are routinely used by Drs Mori and Senis. Functional assays will include aggregation, secretion, adhesion, spreading and flow adhesion. Flow cytometry will be used to measure surface glycoprotein expression and integrin activation in control and mutant platelets. Intracellular tyrosine phosphorylation will be analysed by immunoprecipitation and western blotting. Many of these techniques are commonly used in academic, clinical and industrial biomedical research labs.
Oesophageal cancer remains a tumour with very poor survival. Recent local data shows that 1 and 5 year mortality are 59% and 86% respectively. There is therefore an urgent need to increase our understanding of pathogenesis in order to develop new treatment strategies.

Iron regulation is an important feature of carcinogenesis in colorectal and oesophageal adenocarcinomas, where iron content in colonic and oesophageal epithelium, together with expression of iron import proteins relates directly to cellular proliferation and progression from dysplasia to cancer.

In the lung the key effect is from IRP2, an iron regulatory protein and the effect is strongest in adenocarcinomas.

Our hypothesis is that iron regulation is a driver of progression of oesophageal dysplasia to invasive carcinoma, an effect likely to be more marked in adenocarcinomas with a background of metaplasia (Barretts oesophagus) in the majority compared to squamous carcinomas of the oesophagus.

This project will compare IRP2 expression between normal, dysplastic and cancerous human oesophageal tissue, and test human oesophageal cancer cell lines for IRP2 expression with a view to use in subsequent mechanistic studies.

There will be an option to perform functional studies, such as IRP2 knockdown, if the initial phases of the project go well. In addition, there are options to extend the project into lung cancer.

**Techniques to be used in the project:**

Immunohistochemistry, cell culture, Western blot, qPCR

**Key References:**

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<table>
<thead>
<tr>
<th>Lead Supervisor:</th>
<th>Ms Olga Tucker</th>
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<tr>
<td>Co Supervisor</td>
<td>Dr Alice M Turner  Dr Chris Tselpsis</td>
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<tr>
<td>Project Title:</td>
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<tr>
<td>Contact Email:</td>
<td><a href="mailto:o.tucker@bham.ac.uk">o.tucker@bham.ac.uk</a></td>
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Iron regulation is an important feature of carcinogenesis in colorectal and oesophageal adenocarcinomas, where iron content in colonic and oesophageal epithelium, together with expression of iron import proteins relates directly to cellular proliferation and progression from dysplasia to cancer.

In the lung the key effect is from IRP2, an iron regulatory protein and the effect is strongest in adenocarcinomas.

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**How are you planning to ensure adequate supervision?**

Each of the named supervisors will meet with the student regularly to discuss results and assess progress. Other meetings may take place at the request of either the student or the Mentor.

Existing members of the research team will provide expert training, and the student will be supervised on a daily basis by Paul Newby (technical supervision). The techniques to be used in this project are already established in the laboratory where the project would take place.

Dr Turner has submitted an additional Intercalated BMedSc in Clinical Sciences Research proposal for an in vitro lung cancer cell project. If our proposals are successful the two students would share protocols for the functional work, and provide peer support to each other in addition to the support provided to them by the laboratory staff and supervisors.

**The student role.**

The student will be expected to acquire the basic and more advanced laboratory skills to complete the project.

The student will be required to attend weekly laboratory meetings, and present at the Oesophagogastric Research meeting held on a monthly basis in the CRUK Institute.

The student will be expected to attend a wide range of lectures and interact with other groups in the School of Cancer Sciences to give them breadth of experience and collaboration.
Histones and many non-histone proteins are modified by attachment of acetate groups to selected lysines. Sometimes the modification alters the protein’s function. The targeted acetylation of histones either directly or indirectly alters their association with genomic DNA and can affect gene expression. Histone acetylation is a dynamic process with acetyltransferases (HATs) and deacetylases (HDACs) targeting histones and causing acetate groups to turn over rapidly. Molecules that inhibit the activity of HDACs (HDACi) have been known for many years and have been used experimentally to induce histone hyperacetylation in cultured cells and other model systems, and to explore the effects on cell physiology. More recently HDACi have been used therapeutically for the selective killing of tumour cells. Several are in clinical trials and two have been FDA approved for treatment of specific lymphomas. A key question is why growth of some tumours (or some cell types) is effectively suppressed by HDACi while others seem unaffected.

Our recent work has shown that while HDACi induce global histone hyperacetylation, the histones packaging most genes show little or no change. Genes seem to be sheltered from the effects of HDACi. Further, we have shown that normal cells respond rapidly to HDACi (within 30 min) by changing expression of a selected group of genes, mostly involved in chromatin structure and function. Many HDACi are naturally occurring compounds, and we have suggested that this response is an ancient, evolutionarily conserved mechanism that allows cells to survive environmental HDACi. Over longer times, cells seem to adapt to the presence of HDACi, with loss of histone hyperacetylation and restoration of growth.

This project will explore the mechanisms by which cells adapt to HDACi. Specifically, the experiments will test the hypothesis that adaptation involves the up-regulation or altered function of a group of HDACi-resistant HDACs, namely the Sirtuins, or Class III HDACs. These enzymes are unusual lyases in that they are NAD dependent and their activity is regulated by the redox state (NAD/NADH ratio) of the cell. The proposed experiments will explore both the expression of the Sirtuins (SirT1-6) in response to HDACi using RTQ-PCR, and monitor HDACi-induced changes in NAD/NADH ratio by specific chemical assays. We will use normal and cancer cells

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<thead>
<tr>
<th>Lead Supervisor:</th>
<th>Bryan Turner</th>
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<tr>
<td>Co Supervisor:</td>
<td>Daniel Tennant</td>
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<tr>
<td>Project Title:</td>
<td>Exploring the epigenetic mechanisms by which normal cells and cancers adapt to the cytotoxic effects of histone deacetylase inhibitors</td>
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<tr>
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<td>Contact Email:</td>
<td><a href="mailto:b.m.turner@bham.ac.uk">b.m.turner@bham.ac.uk</a></td>
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| Project Outline |

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grown in culture, including cultured glioma cells grown in both normal and low oxygen conditions. Most tumours grow under hypoxic conditions and brain tumours provide extreme examples.

Reading


Bradbury CA et al (2005) Histone deacetylases in acute myeloid leukaemia show a distinctive pattern of expression that changes selectively in response to HDAC inhibitors. Leukemia 19, 1751-59

**How are you planning to ensure adequate supervision**

The student will have at least weekly meetings with one or both of the project supervisors. Supervision in the laboratory will be by a senior Research Fellow in the Turner group, Dr. John Halsall, whose own CRUK-funded research is closely related to this project.

**The student role.**

The student will learn to grow cells in culture, initially human lymphoblastoid cell lines (our standard model cells), treat them with HDACi (initially sodium valproate, VPA) and monitor the effects on cell growth, cell cycle progression and apoptosis (both by FACS) and induction of histone hyperacetylation (by electrophoresis and western blotting). The ability of cells to adapt to growth in HDACi will be monitored by monitoring the above criteria at different VPA concentrations over periods of several days. Changes in expression of SirT1-6 as cells adapt will be followed by RTQ-PCR, while changes in NAD/NADH will be monitored by chemical assays. Having established the inhibitor treatments and various assays in our model (normal) cells, we will apply them to the more precious glioma cells, grown under both normoxic and hypoxic conditions.
**Lead Supervisor:** Dr Hanene Ali-Boucetta  
**Co Supervisor** Prof Nicholas Barnes  
**Project Title:** *Drug Delivery using Carbon Nanotubes for the Treatment of Multiple Myeloma*  
**Department:** Pharmacy, Pharmacology & Therapeutics  
**Contact Email:** h.aliboucetta@bham.ac.uk  
**Telephone:** h.aliboucetta@bham.ac.uk x 48162  
**Is the project cancer related?** Yes  

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**Project Outline**

New approaches for the treatment of multiple myeloma (MM); a B-cell malignancy, are highly required due to the fact that most patients with MM eventually relapse after treatment. Herein, we will investigate the use of novel delivery vectors for the delivery of drugs used in the treatment of MM such as doxorubicin and thalidomide. Carbon nanotubes (CNT) are graphene sheet layers rolled into a tubular structures and have shown great promise as for the intracellular delivery of small molecules, protein and nucleic acids. CNT will therefore be used for the delivery of mainly doxorubicin but also other drugs used in the therapy of MM in attempt to bypass any acquired drug resistance and deliver therapeutic concentrations of drugs to MM cells. This novel drug delivery system therefore may provide new therapeutic startegies for the treatment of MM. The project will involve the:

- Preparation of the carbon nanotubes (CNT) dispersion
- Preparation of the CNT: Doxorubicin complexes
- Preparation of CNT: Thalidomide complexes (depending on the time)
- Characterisation of the CNT: drug complexes using spectroscopy and microscopy techniques
- Assessment of the uptake of CNT: drug complexes inside the cells.
- Assessment of the efficacy of CNT: drug complexes *in vitro* in comparison to drug alone (apoptosis and cell death assays)
- The use of cell lines with relative resistance to drug impact will establish the potential benefit of using a delivery vector.
**How are you planning to ensure adequate supervision?**

- Regular meetings with the student in a daily and weekly basis to discuss the science and any other issues.
- Open door policy in case the student has any issues to report.
- Provide the student with regular feedback on his/her progress.
- Assess the student in his/her laboratory work and training as well as their written project.

**The student role.**

- Performing the experiments in the lab under direct supervision at the beginning of the project and as the time passes the student will become more independent.
- Preparation of the carbon nanotubes (CNT) and the CNT: Drug complexes
- Characterisation of the CNT: Drug complexes
- Assessment of the efficacy of the drug alone and CNT: Drug complexes.
- Incubating the cells with CNT and CNT: Drug complexes
- Performing different toxicological assays to assess the efficacy of the drug once delivered by the delivery vector (CNT).
- If time allows, the student will also investigate the possibility of the complexation of thalidomide with CNT and assess the therapeutic efficacy of this novel complex.
Lead Supervisor: Dr M.-C. Jones

Co Supervisor: Professor N.M. Barnes

Project Title: Investigation of the impact of nanoparticles upon human normal and pathological cells

School/Section: School of Clinical and Experimental Medicine, Section of Pharmacy, Pharmacology and Therapeutics

Contact Email: m.jones.8@bham.ac.uk
Telephone: 48188

Is the project cancer related? Yes

Discipline:
- Cancer Sciences
- Pathology
- Metabolic Medicine
- Haematology
- Infection
- Immunology
- Anatomy
- Endocrinology
- Liver & GI Medicine
- Evolutionary biology in Clinical medicine

Project Outline

Knowingly or not, most people are exposed to nanosized material (defined as being between $1 \times 10^{-9}$ and $1 \times 10^{-6}$ m in size) on a daily basis. The potential impact of these nanoparticles on human health has been the focus of environmental and occupational health sciences. Concentrating mostly on inorganic nanoparticles, these studies have identified particle characteristics such as surface properties, dose and composition that may be related to adverse reactions, mostly inflammation. These studies aimed mainly at defining guidelines on the safe use of nanoparticles.

In parallel, nanoparticles made of so-called organic "soft-material", such as lipids or polymers are increasingly being evaluated as tools for medical applications. Their small size has been shown to provide several advantages that allows for targeted accumulation of the carrier and its load at a specific site. Yet, little is known of the activity, toxicity or inflammatory potential of the carriers themselves upon normal or pathological human cells. Although the data available so far tends to support their safety, some properties such as surface charge are known to impact on their behaviour and fate in the body. Furthermore, few studies have compared different nanoparticles under set test conditions upon exposure to normal and pathological cells (e.g. heamatological cancer cells), making it difficult to draw definite conclusions as to whether one type of particle should be preferred over another as a medical device.

Aiming to build on the current knowledge and understanding of nanoparticle-cell
interaction, this project will focus on soft-material nanoparticles such as those commonly used in pharmaceutical nanotechnology. Experiments will concentrate on internalisation, toxicity and pro-inflammatory potential on ‘normal’ and pathological human cells.

To this end, nanoparticles which may vary in size, charge or composition will be incubated with primary or immortalised human cells in culture (typically human immune cells and their malignant counterparts). Using common techniques, exposed cells will then be assessed for 1) changes in the expression of biochemical markers (e.g. production of cytokines) 2) changes in cell integrity (e.g. toxicity, apoptosis) 3) changes in cell function (e.g. phagocytosis function) 4) extent of nanoparticle internalisation.

Experimental conditions will be varied in order to evaluate the effect of parameters such as particle dose and exposure time. Cell internalisation pathways and may also be probed.

How are you planning to ensure adequate supervision?

Active support will be provided at every step starting from experimental design to data analysis, as required, by

1) Providing adequate facilities for the work to be conducted
2) Clearly defining the objectives of the project
3) Identifying supervision needs
4) Providing sufficient background literature to put the project in context
5) Providing support in experimental design
6) Providing technical support as required during the experiments
7) Providing guidelines for data analysis and report writing
8) Arranging regular meetings to follow progress
9) Making sure any arising issues are addressed in a timely fashion

The student role.

The student is expected to conduct cell culture experiments and be trained in using the different techniques required for completion of the project. The student will acquire some knowledge on nanoparticles for pharmaceutical applications. The student is also expected to provide updates on project progression and keep the supervisors informed of any difficulties encountered.
**Lead Supervisor:** Z Nagy

**Co Supervisor** R Bicknell, G Brown

**Project Title:** Effect of Retinoic Acid Receptor Antagonists on Neurones and Angiogenesis

**Department:** CEM

**Contact Email:** z.nagy@bham.ac.uk

**Telephone:** 58135

**Is the project cancer related?** Yes

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**Project Outline**

Neurones play a role in Alzheimer’s disease and endothelial cells in angiogenesis and tumour growth. Both neurones and endothelial cells have active retinoic acid receptors (RAR’s).

Retinoids are naturally occurring compounds that are structurally related to vitamin A. **All-trans** retinoic acid (ATRA) is the biologically active metabolite of vitamin A. It has been known for many years that ATRA, and other retinoids, play an important role during the development of an organism, by orchestrating pattern formation and by regulating the differentiation, proliferation and survival of many types of cells [1]. Retinoids mediate these biological effects by activating one or more of the closely related retinoic acid receptors (RAR) – there are three main sub-types RARα, RARβ, and RARγ. These receptors function as ligand-dependent transcriptional regulators and form heterodimers with retinoid X (or rexinoid) receptors (RXRα, β, and γ). The dimers bind to retinoid response elements (RAREs) that are located in the promoter region of target genes to stimulate gene transcription [1]. Retinoids are used in the treatment of certain types of cancer (lymphomas, leukemias, neuroblastoma) and have been proposed as a possible treatment for Alzheimer’s disease [2].

Some insight to the particular function(s) of each of the sub-types of RAR has been obtained from studies of the development of blood cells. It is well known that liganded RARα drives the differentiation and maturation of primitive haematopoietic cells. The function of RARα is particularly interesting as this receptor appears to be essential to expansion and survival of haematopoietic stem cells. Mice in which the
RAR- receptor has been knocked-out have a reduced number of haematopoietic stem cells.

To investigate the function of each of the sub-types of receptor we have produced a panel of compounds that can be used to switch-on (agonists) or switch-off (antagonists) individual receptor sub-types. The compounds are highly selective and have a very high affinity (a few nM) for the appropriate receptor sub-type. **The agonist and antagonist of RAR- are uniquely available to our group.** Other agonists and antagonists are some of the best available as to sub-type receptor specificity and binding affinities.

We propose to further characterise the effect of these compounds on cell proliferation, differentiation, cell death and cell type specific functions in both neuroblastoma cells and endothelial cells. In neuroblastoma cells we will concentrate on the effect of these compounds on the expression, processing and cellular localisation of neurone-specific proteins (tau, APP, alpha synucleine). In endothelial cells we shall investigate effects on cell migration and tube formation.

The high content cytometry (figure 1) technology (ACUMEN, TTP Labtech) available to us (**we are the only University in the UK to have access to this technology**) allows the in depth analysis of the cellular changes induced by these compounds.

The compounds with promising anticancer and antiangiogenic properties *in vitro* will be further tested in *in vivo* models, such as zebrafish genetically engineered to have green flourescent blood vessels (figure 2) and the sponge angiogenesis assay (figure 3) in mice.


**How are you planning to ensure adequate supervision?**

Both the Nagy and Bicknell labs have several people trained to use the techniques necessary for this project. The supervisors will meet with the student at least once a week to assess progress. The student will be expected to actively participate in weekly lab meetings, including the presentation of their own work.

**The student role.**

The student will be in charge of the project. They will carry out the experiments, analyse the results and write their dissertation. Although guidance and support will be provided at each step as necessary, the project, a collaboration between the three groups, will be executed to completion by the student. The supervisors plan to submit a paper based on the evidence collected by the student to complement the data collected previously on these compounds. Since the compounds are unique to us and the technologies used for the completion of the project are state of the art, the possibility of producing novel findings is high.
Lead Supervisor: Dr Francis Mussai

Co Supervisor: Dr Vicki Weston/ Dr Pam Kearns

Project Title: Investigating the role of CAT arginine transporters in Acute Myeloid Leukaemia

Department: School of Cancer Sciences

Contact Email: Francis.mussai@bch.nhs.uk
Telephone: 07919 174479

Is the project cancer related? Yes

Discipline: Cancer Sciences Immunology
Pathology Anatomy
Metabolic Medicine Endocrinology
Haematology Liver & GI Medicine
Infection Evolutionary biology in Clinical medicine

Project Outline

Background: Acute Myeloid Leukaemia is the most common acute leukaemia of adults and the second most common leukaemia of childhood. Despite significant advances in therapy the prognosis for the majority of patients remains poor.

We have previously demonstrated that AML blasts over-express arginase II and iNOS which metabolise and deplete arginine from the microenvironment. As a result AML creates an immunosuppressive micronevironment leading to inhibition of T cell proliferation, modulation of bone marrow macrophages to an M2-immunosuppressive phenotype, and suppression of haematopoiesis from murine GMPs and human CD34+ HSCs. (Submitted for publication)

Although AML blasts deplete the microenvironment of arginine, we have preliminary evidence that AML blasts are resistant to the low nutrient microenvironment. Arginine is transported into non-malignant myeloid cells through a family of transmembrane substrate transporters - the Cationic Amino Acid Transporters (CAT). However to date the role of CAT transporters in Acute Myeloid Leukemia has not been investigated,

Hypothesis: Acute Myeloic Leukaemia blasts over-express CAT family proteines to sequester arginine from the microenvironment.

Aims:
- To characterise the CAT expression profile in AML cell lines and patient-derived blasts by flow cytometry, Western blot, and RT-PCR
- To understand the role of cytokines in CAT expression
- To gather preliminary evidence on the role of CAT inhibition on AML blast viability and suppressive phenotype

Methods overview:
- Tissue culture of AML cell lines/ patient samples
- Analysis of CAT isoforms expression by AML cell lines using RT-PCR and Western blot
- Investigation of the role of cytokines present in the AML microenvironment on CAT expression (e.g. IL-1, SAA) through in vitro culture assays followed by RT-PCR/Western
- Use of CAT inhibitors (e.g. Nethylmaleimide) to investigate the effect of CAT blockade on AML viability, response to chemotherapy, and arginine metabolism
- Mixed Lymphocyte Reaction in the presence of AML blasts

**Significance:**
This study is readily achievable within the time-frame for a motivated BMedSci student. The results of the study will provide new insights into AML immunobiology and have the potential to identify new therapeutic targets in AML. The study data will contribute to ongoing AML studies in the group, and likely form part of a future publication.

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**How are you planning to ensure adequate supervision?**

Overall supervision of the project will be undertaken by Dr Francis Mussai, with at least weekly meetings with the student to review data.

The student will work closely with senior post-doctoral Fellows Dr Vicki Weston and Dr Carmen DeSanto on a daily basis to become proficient in the laboratory techniques required, and for trouble-shooting.

Data will be reviewed in joint lab meeting between the Mussai and Kearns groups.

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**The student role.**

The student will learn to perform core laboratory skills and data analysis. He/she will be guided through the experiments by senior post-doctoral Fellows and will take shared responsibility for the data generated.

He/she will become a key member of the group during the placement and will have the opportunity to understand the process of laboratory science. The project is likely to generate novel and relevant data that can be used in future studies and publications, for which the student will be given recognition. The student will be able to write the data up into an interesting thesis.
Relapsing-remitting multiple sclerosis (RR-MS) is considered to be an autoimmune disease, with both human studies and animal models suggesting that autoreactive T cells target components of the myelin sheath, with secondary damage to neuronal tissues. The pathophysiology of pathogenic T cells in RR-MS has been an intense area of research over the past decade, with significant advances in our understanding of the underlying molecular and cellular biology. Although both CD8+ and CD4+ T cells have been implicated in the disease processes, the majority of studies have focused on the nature of the CD4+ T helper (Th) cell subsets, and in particular the Th17 cellular subset.

Th17 cells are not only characterised by the expression of IL-17, but also express the transcription factor RORC (RORγt) and the surface receptors CD161 and CCR6. We have recently identified a population of Th1 IFN-γ-secreting CD4+ T cells that also express CCR6 and RORC. There are many questions that remain to be defined about these cells including:

1. Are these cells found in increased numbers in the blood or cerebrospinal fluid of patients with RR-MS, and does this relate to disease relapse?
2. Why do these cells not express IL-17?
3. What is the antigen specificity of these T cells – do they recognise myelin antigens?

These questions form the basis of much of the research in the group around RR-MS. The project available to you will be around addressing one of the key questions above, depending on progress that has been made by the group and advances in the literature.

The techniques used in the laboratory include cell purification, using fluorescence-activated cell sorting and magnetic bead isolation, cell culture, multi-colour flow cytometry and real-time RT-PCR.

References

How are you planning to ensure adequate supervision?

The student will receive extensive training from both Dr Curnow’s research technician and other members of the research group. The experimental design, analysis of data and interpretation will be developed at regular meetings with Dr Curnow (at least weekly). The group holds research meetings once a week where updates of projects are presented on a rotating basis. Dr Douglas (consultant neurologist) will also participate in discussions on a regular basis.

The student role.

The student will be expected to liaise with both the supervisor and other members of the Curnow group to ensure that experiments are appropriately designed, implemented and analysed. The student is ultimately responsible for carrying out the laboratory experiments, and may also be involved in the collection of clinical samples where appropriate.

The project will be designed to produce robust and reproducible results addressing one of the key questions outlined above. This will therefore represent an ideal opportunity for the student to contribute to an exciting and fast moving area of research in MS, and autoimmunity/inflammatory disease in general.
Chronic lymphocytic leukaemia (CLL) is a malignancy of mature B lymphocytes that accumulate in patients’ peripheral blood, bone marrow and lymph nodes. Although reduction in tumour load can be achieved under current treatments, the disease is incurable. The biological course of CLL is extremely variable and influenced by many factors, including the status of B-cell receptor (BCR) signaling and DNA damage response. An emerging concept suggests that CLL cells in peripheral blood (PB) are dormant, whereas CLL cells in lymphoid organs actively proliferate. It is believed that CLL is driven by complex interactions with the microenvironment and activation of BCR signaling, possibly via antigen stimulation. BCR signaling is initiated upon ligation of the BCR receptor to a ligand that triggers a wave of cellular responses including phosphorylation of the Src family kinases, Lyn and Syk, formation of a signalingosome containing the kinases Syk, Btk, Lyn and adaptor proteins Grb2 and BLNK, activation of secondary messengers, calcium influx and subsequent activation of the NFkB, Akt, Ras and MAPK pathway. The collective effect of these responses is increased survival and proliferation of B cells.

Accordingly, the BCR signaling cascade has emerged as a new therapeutic target and range of new molecules targeting different points are under pre-clinical or clinical testing. Unfortunately, access to lymph nodes, the site of BCR activation, is limited and therefore pre-clinical data on the comparative efficacy of different BCR inhibitors in CLL is insufficient. Therefore, there is an urgent need to develop systems in which these new agents that target the BCR cascade can be reliably tested.

We have recently developed three xenograft models for primary CLL on the NOG background and have shown that CLL cells with different biological features (stage of disease, VH status, ATM and TP53 status), can be successfully initiated to proliferate. Furthermore, we have shown that these models can be used to address the cytotoxic impact of novel therapies. Similarly, we have optimized an in vitro system where CLL cells can be initiated to cycle in the presence of CD40L expressing murine fibroblasts and IL-21. However, despite our ability to initiate proliferation of CLL cells both in vitro and in vivo, the capacity of these systems to induce BCR signaling and mimic CLL engagement in lymph nodes or bone marrow is yet to be determined. Therefore, the aim of this study is to determine the ability of our in vitro and in vivo systems...
 systems to mimic CLL engagement and activation of BCR signaling in proliferative centers in lymphoid organs. The student will compare BCR signaling in CLLs with different profiles of DNA damage response, initiated to cycle in a supportive microenvironment.

The first task will be to determine whether our in vitro and in vivo systems induce BCR activation compared to non-proliferating cells. Using magnetic beads and positive selection the student will purify CLL cells following culture in vitro (in the presence of autologous T cells, CD40L and IL-21) or upon their engraftment in the spleens/bone marrow of NOG mice. Assessment of BCR activation will be performed by three complementary approaches a) quantitative RT-PCR for well established signature genes activated by BCR signaling b) FACS analysis to measure activation (phosphorylation) of principal BCR signaling proteins (Syk, Akt, Erk and Mek) and c) immunohistochemistry for phosphorylated Akt, Erk and Mek. Comparison will be made between tumour cells from the peripheral blood of CLL patients and the same cells following either engraftment in the xenograft model or in vitro culture. A comparison will also be also made between three CLL xenograft models a) CLL engraftment in bone marrow and spleen of mice with previously humanised haemopoiesis b) CLL engraftment in the presence of activated autologous T cells c) engraftment in the presence of allogeneic monocytes.

The second aim will be to compare BCR induction in CLL cells with different ATM and TP53 genotypes in the various culturing and engraftment regimes. Targeting tumours that harbour mutations in p53 pathways that are resistant to the classic DNA damaging agents and identifying the best system to test response of these tumours to novel agents will be of particular importance.

References:


How are you planning to ensure adequate supervision?

All proposed experiments are routinely performed in our laboratory by two post-docs (co-supervisors). The first two weeks student will shadow the co-supervisors and in the third week he/she will initiate independent experiments under supervision. The main supervisor will communicate with the student on the daily basis to establish the plan of the project and follow its progress, and this will be reinforced during weekly group meetings. Four weeks before the end of experimental work, a plan for the write up will be agreed.

The student role.

The student will be expected to follow instruction, adhere to the weekly plans, and report results at the group meetings. He/she will be expected to communicate any uncertainty regarding the project and seek clarification and advice.
Lead Supervisor: Gideon Hirschfield

Co Supervisor: Evaggelia Liaskou

Project Title: The Role of TNFRSF14-BTLA Pathway in The Pathogenesis of Primary Sclerosing Cholangitis

Department: Immunity & Infection, Centre for Liver Research

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Is the project cancer related? No

Discipline: Cancer Sciences Immuno-pathology
Pathology
Metabolic Medicine
Haematology
Infection

Immunology
Anatomy
Endocrinology
Liver & GI Medicine
Evolutionary biology in Clinical medicine

Project Outline

Primary sclerosing cholangitis (PSC) is a chronic liver disease characterised by progressive inflammation, fibrosis and destruction of the bile ducts, eventually leading to end-stage liver disease. PSC has the greatest unmet need in Hepatology, with the majority of patients ultimately needing liver transplantation to avoid death from liver failure. Thus, understanding the disease etiopathogenesis is an urgent need. The cause of PSC has not been defined, however high periportal mononuclear cell infiltration has been found in most PSC liver biopsy specimens, with at least 80% being T lymphocytes. Interestingly, a reversal of the CD4/CD8 ratio (1:2) in PSC liver has been reported when compared to autologous peripheral blood (3.5 : 1). Additional findings report abnormalities in the lymphocyte subsets, activation status and presence of autoreactive T cells, which collectively support the belief that T cells are critical for the disease progression. The most significant recent discovery in PSC research has been the identification of specific genes associated with disease, and TNFRSF14 has been recognised as a robust novel risk locus with PSC.

TNFRSF14 is a receptor that belongs to the TNF superfamily, it has a wide tissue distribution and cell type expression. TNFRSF14 is expressed on both CD4 and CD8 T cells and is known to activate cell survival genes through NF-kB transcription factors. There are two types of cellular ligands for TNFRSF14: 1) the TNF-related cytokines, LIGHT and lymphotoxin-alpha and 2) the immunoglobulin superfamily members BTLA (B and T lymphocyte attenuator) and CD160. BTLA is an inhibitory coreceptor expressed on lymphocytes that plays crucial role in maintaining immune homeostasis. Interaction of BTLA and TNFRSF14 causes attenuation of IL-2 production and thus reduction of T cell proliferation. Studies have reported that co-expression of TNFRSF14 and BTLA in T cells creates an intrinsic mechanism that interferes with the ability of LIGHT and the other ligands to access and activate TNFRSF14. Interestingly, studies in BTLA deficient mice have revealed that lack of BTLA led to spontaneous development of autoimmune hepatitis-like disease characterized by increased numbers of activated CD4+ T cells, severe bile duct epithelium inflammation and irregularity of duct outlines, paralleling features of histopathological liver biopsy specimens in human PSC. Collectively, these reports suggest the important role of BTLA in maintenance of immune tolerance and prevention of autoimmune diseases.

Our immunohistochemical characterisation of TNFRSF14 in human liver has
revealed its expression in lymphocytic infiltrates (Fig1A) as well as in hepatic sinusoids (Fig1B) and biliary epithelial cells (Fig1C).

**Figure 1.** Immunohistochemical analysis of TNFRSF14 expression in human PBC liver. TNFRSF14 is expressed in A) lymphocytic infiltrates, B) hepatic sinusoids (Kupffer cells/macrophages and/or sinusoidal endothelial cells) and C) biliary epithelial cells.

The aims of this project are to fully characterise the expression of BTLA and TNFRSF14 in human PSC liver in comparison with PBC and other chronic liver inflammatory diseases. In particular,

the aims are:

1. Study the total (protein and RNA) expression of BTLA in human PSC and PBC liver and compare with healthy donor and other chronic liver inflammatory liver diseases. (A reduction in BTLA expression might predispose susceptibility to autoimmunity)

2. Study the expression of BTLA in the different subsets of liver infiltrating lymphocytes i.e CD4, CD8, Tregs derived from PSC, PBC, normal, alcoholic liver disease livers. Compare the expression levels with lymphocyte subsets from peripheral blood of patients with PSC and other chronic liver inflammatory diseases.

3. Study the expression of TNFRSF14 in the different subsets of liver infiltrating lymphocytes i.e CD4, CD8, Tregs derived from PSC, PBC, normal, alcoholic liver disease livers. Compare the expression levels with lymphocyte subsets from peripheral blood of patients with PSC and other chronic liver inflammatory diseases.

**How are you planning to ensure adequate supervision?**

There will be a weekly meeting for at least an hour in order to ensure the efficiency of student and progress of the project. Introduction to the laboratory techniques and day-to-day supervision will be provided by post-doc Dr Liaskou.

**The student role.**

The role of the student will include to:

1) Perform western blot and real-time PCR analysis for detection of protein and RNA levels, respectively of both BTLA and TNFRSF14 in human liver diseases. This will include learning of preparation of protein lysates as well as RNA extraction and cDNA synthesis prior to western blotting and quantitative PCR

2) Isolate liver infiltrating mononuclear cells (LIMCs) from normal donor, resected and transplanted human liver.

3) Phenotypically characterise LIMCs for the expression of TNFRSF14 and BTLA by flow cytometry.
**Lead Supervisor:** Andrew Turnell

**Co Supervisor**
Ashley Martin

**Project Title:** Proteomic analysis of UbcH10 in human cancer cell lines

**Department:** Cancer Sciences

**Contact Email: Telephone:**
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Martin 49531 A.Martin@bham.ac.uk

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**Is the project cancer related?** Yes

**Project Outline**

The E2 ubiquitin-conjugating enzyme, UbcH10 works in conjunction with the anaphase-promoting complex to promote cell cycle progression through mitosis. UbcH10 is overexpressed in a number of human cancers and promotes chromosome missegregation, aneuploidy and tumour progression. Given these findings, UbcH10 is a target for therapeutic intervention. However, despite its recognized role in cancer pathogenesis very little is known about how UbcH10 is regulated at the molecular level.

The initial aim of this project is to map the UbcH10 interactome using a combination of immunoprecipitation and mass spectrometry. The interactome can be defined as the proteins that associate (interact) with a protein in the cell under a defined set of conditions. Elucidation of the interactome of proteins whose role is unknown has proven to be a very useful way of determining functionality. New UbcH10-binding proteins will be validated by conventional immunoprecipitation/Western blot analyses. The precise experiments that follow this characterisation will depend on which proteins are found to interact with UbcH10 but any such experiments will be designed to dissect the way in which UbcH10 functions. Also, given that post-translational modification is known to regulate cell cycle progression we will also use mass spectrometry techniques to determine whether UbcH10 is post-translationally modified by phosphorylation and/or acetylation during the cell cycle. These analyses will help identify UbcH10-binding proteins and post-translational modifications that might regulate UbcH10 activity and contribute towards cancer pathogenesis.
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<th><strong>How are you planning to ensure adequate supervision?</strong></th>
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<td>Dr Turnell will provide all of the supervision with regards to the “cell biology and virology” aspects of the project. He has considerable experience in this work and will provide clear instruction/training for the cell culture work required in addition to the biochemical work required. This will include all of the immunoprecipitation protocols required to generate interactomic data. Dr Martin will provide the training/supervision for the proteomic analysis of the samples generated in the project. This will include the preparation of samples for analysis using the proteomic protocols developed in his laboratory in addition to operating the HPLC/Mass spectrometers for analysing the samples. Full training in data mining programs will be provided as will the procedures required for interpretation of the results obtained. Dr Turnell and Dr Martin have collaborated for several years on “interactomic” projects and are very experienced at training/supervising students in all facets this work. These previous collaborations have resulted in several high quality publications. The complementary experience of the 2 main supervisors will ensure that the student receives expert input in all of the techniques utilized. Furthermore, Dr Turnell has experienced members of his group that will provide “on spot help” as and when required as will members of Dr Martin’s proteomic group. Both supervisors will have regular contact with the student during the work at the level of day to day supervision and formal meetings will be held every 2-3 weeks to monitor progress. Feedback from the student about the quality of training will be taken at these meetings and any changes required will be carried out.</td>
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<th><strong>The student role.</strong></th>
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<td>The Student will be trained in cell culture work in order that they can prepare their own samples for analysis. Also the cell harvesting and processing procedures will all be completed by the student such that they will receive a thorough training in basic and complex protein biochemistry protocols. The immunoprecipitation procedures required for mass spectrometry based analyses are more rigorous than normal immunoprecipitation protocols and complete training will be provided. Once suitable samples and relevant controls are produced the proteins will be resolved using SDS PAGE, stained and bands of interest excised and treated with trypsin. The tryptic peptides generated will be separated by reverse phase HPLC and sprayed on-line directly into an ESI source on a ToF/ToF mass spectrometer. The mass spectrometer will perform an automated MS MS/MS procedure so that the peptides are fragmented and can be identified. The student will be shown how to do this and how to search the data to identify the proteins and to assess the quality of the data. Therefore, the student will be responsible for all aspects of the work and in doing so will be fully trained in a range of fundamental techniques that will be widely applicable in future work.</td>
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Antibiotic resistance is one of the great global health challenges of the 21st century and a problem which is rapidly worsening with the emergence and spread of multidrug resistant pathogens and a worrying dearth of new drugs in development. This has raised the real scenario of infections with untreatable pathogens and threatens an extraordinarily broad range of clinical activities where antibiotics are needed to both treat infection and provide prophylactic cover to allow complex surgery etc.

Bacteria can employ a range of mechanisms to resist the actions of antibiotics, recently we have discovered that mutations within DNA gyrase which confer resistance to the powerful quinolone antibiotics by altering their target site also give a low level of resistance to many other drugs which do not target this enzyme. DNA gyrase controls how DNA is packaged (supercoiled) within the cell and changes in its activity alter chromosome structure and as a result expression of many other genes. We have found that gyrase mutants show up-regulation of stress response pathways as a result of altered supercoiling which we hypothesise to provide the broad antimicrobial protection seen and may help explain why these mutants are commonly seen in pathogenic bacteria.

In this project we aim to test our hypothesis by artificially inducing supercoiling changes and: determining the effect on expression of stress response pathways and determining the effect on antibiotic sensitivity.

We also aim to evaluate how broadly conserved this mechanism of resistance is amongst bacteria, our preliminary work has all bee with the model food borne pathogen *Salmonella*, we will evaluate whether *E. coli*, a major pathogen for which quinolone resistance is common behaves in a similar manner and use in-vitro evolution competition experiments to determine the advantage gyrase mutants may have when exposed to non quinolone drugs and compared to wild-type strains.
### How are you planning to ensure adequate supervision?

Students will meet weekly with Dr Webber and will be supervised daily by Dr Webber or other members of the Webber laboratory. There are currently 17 researchers in the antimicrobials research group ensuring appropriate cover will be available for student supervision. Webber has successfully supervised five intercalating students in the last four years.

### The student role.

The student will be responsible for performing and analysing experiments under the direction of Dr Webber and will be treated within the laboratory as any other member of the research team. The student will have responsibility for investigating the background of the project and developing a good awareness of the context and aims of the project.
Project Title: Profiling Stem Cell Epigenetic transitions within a hematopoietic stem cell niche.

Department: Immunity and Infection

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Telephone: 0121 4146810

Is the project cancer related? Yes

Discipline:
- Cancer Sciences
- Pathology
- Metabolic Medicine
- Haematology
- Infection
- Immunology
- Anatomy
- Endocrinology
- Liver & GI Medicine
- Evolutionary biology in Clinical medicine

Project Outline

Emerging data from a number of experimental systems indicates that stem cell function is critically influenced by signals derived from the microenvironment or “niche.” The organisation within a niche and cues from surrounding stromal cells is key to controlling the balance between stem cell self-renewal and differentiation. The paired hematopoietic organs of the Drosophila genetic model organism offer a well-characterized system in which the regulation of stem cell behavior within niches can be genetically dissected. This system allows insight into how stem cell self-renewal and differentiation can be influenced by signals from stromal cells in the niche, and integrated with hormonal and endocrine signals. This is critical to understanding the mechanisms leading to aberrant stem renewal and differentiation in cancers and leukemias.

Research using cultured stem cell lines shows that stem cell differentiation is accompanied by alterations in the chromatin architecture, with stem cells exhibiting unique patterns of histone post-translational modifications (so-called “epigenetic marks”). In vitro tissue culture systems, however, do not allow the influence of signals from the niche on the establishment and propagation of these patterns of epigenetic modifications to be dissected. The extraordinary genetic amenability of Drosophila offers a system in which targeted ablation or over-expression of signalling mediators allows experimental manipulation of signals from the niche to the stem cell populations to investigate effects on epigenetic landscapes. To date, however it has not been possible to generate experimentally sufficient quantities of chromatin from these cell populations. Here we have developed techniques to allow purification of chromatin of stem cell populations from in vivo stem cell niches for profiling using the powerful genomics technique of ChIP-sequencing.
We have fly strains that allow tissue specific over-expression of histone H2B variants that contain a recognition sequence for the biotin ligase BirA. This allows in vivo labeling of histone H2B (and thus chromatin) with biotin and purification of nucleosomes using streptavidin-coated beads. We will generate Drosophila strains in which BirA-substrate H2B is expressed in either hematopoietic stem cell populations, lineage-committed progenitors or differentiated leukocyte populations. Chromatin will be crosslinked to DNA, and soluble chromatin prepared from whole animals. It is comparatively easy to generate large numbers of Drosophila quickly and cheaply. Bulk collections will be used as a source of chromatin from which nucleosomes from defined cell populations will be isolated using streptavidin-coated beads. Subsequently, whole genome distribution of histone modifications associated with active gene expression and silent chromatin – the so-called active and repressed marks of Histone H3K4me3, H3K9me3 and H3K27me3 will be determined by chromatin immunoprecipitation coupled sequencing (ChIP-Seq), using modification-specific antibodies. Stem cell, lineage committed and differentiated cell types will be profiled in a first series of proof-of-principle experiments. In the longer term we will characterize alterations that occur within the stem cell populations in response to changes in signals emanating either from the niche (the posterior signaling centre (PSC)), or in response to changes in hormonal/endocrine signals (the fly steroid ecdysone), and in tumour-promoting fly genetic backgrounds.

How are you planning to ensure adequate supervision?

We are a small research-centric laboratory. Supervisors will have daily contact with the student, weekly lab meetings at which data is presented to the group as a whole and in depth monthly one-to-one strategy and revision meetings modelled on the GRS2 format.

The student role.

The student will make extensive use of Drosophila genetics, crossing genetic strains to generate the desired genetic backgrounds to generate marked chromatin for specific cell types in the hematopoietic cell lineages. The student will isolate labelled chromatin from whole animal preparations using magnetic selection procedures. The student will perform chromatin immunoprecipitation to isolate nucleosomes containing specific histone post-translational modifications from the preparations and then prepare purified nucleosomal DNA for high-throughput sequencing on the SOLiD next generation sequencer. The student will map sequence reads to the Drosophila genome using the Bioscope genome analysis pipeline and will determine whole-genome distribution of specific histone marks in cells using ChIP-Seq calling programmes such as MACS. The student will be guided through bioinformatics data analysis of sequencing data.
**Lead Supervisor:** Paul Moss / Guy Pratt

**Co Supervisor**
Helen Parry

**Project Title:** T cell responses in Chronic Lymphocytic Leukaemia

**Department:** Cancer Sciences

**Contact Email:** Hmp050@bham.ac.uk

**Telephone:**

| Is the project cancer related? | Yes |

**Discipline:**

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### Project Outline

Chronic Lymphocytic Leukaemia (CLL) is an incurable B-cell malignancy and many patients die from infection as result of T cell dysfunction and hypogammaglobulinaemia-induced immunosuppression.

Large clonal populations of T cells are commonly found in patients with CLL and are thought to be in response to antigenic stimulation by a limited number of antigens. Surprisingly, T cells also appear to play a role in promoting proliferation of the malignant B cells, suggesting that they may play a role in driving the CLL disease process.

Approximately 70% of patients with B-CLL are infected with cytomegalovirus, a human herpes virus. CMV-specific T cells are found at very high level in patients with CLL and their numbers increase with disease progression. The mechanisms by which this occurs and the specific role of CD4+ and CD8+ T cells in sustaining CLL growth are unknown.

This project will use ‘state of the art’ technology within the research team to examine the kinetics, specificity and phenotype of CMV-specific T cells in CLL. HLA class II ‘tetramers’ will be available for the first time to study human disease. A novel technique known as digital PCR will be used in an attempt to isolate viral DNA and correlate this with T cell immunity.

This work will help towards our understanding of the potential role that CMV infection may play in CLL and may pave the way for the use of antiviral agents during chemotherapy for CLL.

How are you planning to ensure adequate supervision?
Day to day supervision in the lab by Dr Helen Parry. Project supervision with regular meetings with Dr Guy Pratt and Professor Paul Moss. Weekly attendance at ‘Moss group’ meetings and journal club.

### The student role.

- Aim to follow CLL patients from the QE and BHH through treatment and isolate CMV-specific T cells
- Phenotypic analysis of T cell populations to include use of inhibitory T cell ligands such as PD1.
- Optimisation of ‘digital PCR’ technique to detect viral load in patients.
- T cell response and viral load will be correlated with clinical outcome of a patient cohort (30-50 patients)
Background
Platelets play an important role in normal haemostasis to prevent excess blood flow following vascular injury and can be regulated by aggregation, adhesion, secretion or procoagulant activities. Platelet bleeding disorders can present with variable penetrance ranging from mild to severe bleeding. For example Von Willebrand disease is the most common inherited bleeding disorder in which the mild forms are highly under diagnosed. Therefore there are a large number of patients with unclassified platelet bleeding disorders which underlies the need for comprehensive molecular diagnostic tools which will increase the capacity for early and rapid identification of these disorders. The recent advent of whole exome sequencing and next generation technologies has greatly enhanced the probability and speed of identifying mutations and hence causative genes in such conditions. Using this approach we have recently identified a novel gene (ANKRD18A) in affected children with an inherited form of thrombocytopenia (low platelet count) which causes severe bleeding.

We have recently established a cohort of 200 patients with platelet-based bleeding disorders. In this project, you will be trained in the use of next generation sequencing to identify genetic mutations in a small subgroup of these with a clear phenotype, and perform corresponding biochemical studies to verify the defect.

Techniques to be used in the project
Gene identification studies (genetic mapping, bioinformatic analysis, second generation sequencing), functional analysis of mutant gene products (protein expression analysis, Western blotting, cell localisation studies, analysis of downstream target genes).

References
[1] Morgan NV, Goddard S, Cardno TS, McDonald D, et al. (2011) Mutation in the TCRα subunit constant gene (TRAC) leads to a human immunodeficiency disorder characterized by a lack of TCRα+ T cells. J Clin Invest 121(2):695-
How are you planning to ensure adequate supervision?

From a day to day basis I will be directly involved in the laboratory supervision of the student.

The student role.

The student will perform the techniques mentioned above as well as playing a role in defining the clinical phenotype of the inherited platelet-based bleeding disorders. The immediate focus of the planned project will be the identification of new causative genes using the powerful technique of whole exome sequencing. Further investigation of the role of the identified disease-causing mutations/genes will be performed. The precise techniques to be applied will depend on what type of gene is identified and what is already known, as well as the availability of relevant patient material such as cryopreserved peripheral blood mononuclear cells, disease tissue and/or cell lines. Functional analysis of human mutated genes could include cellular transfection and localisation by immunocytochemistry/flow cytometry, and in situ mutagenesis and expression of mutant proteins. Real-time PCR and transcriptional array analysis may be required to investigate expression of the wild type gene or, in the case of a transcription factor, to investigate the effect of mutations on regulatory activity. The project is highly likely to yield novel genes for these platelet-based bleeding disorders leading to a publication.


Project Title: Characterisation of virus-host interactions important for the Merkel Cell Polyomavirus life cycle

Department: Cancer Sciences

Contact Email: j.l.parish@bham.ac.uk
Telephone: 0121 4158151

Is the project cancer related? Yes

Discipline: Cancer Sciences
Pathology
Metabolic Medicine
Haematology
Infection
Immunology
Anatomy
Endocrinology
Liver & GI Medicine
Evolutionary biology in Clinical medicine

Project Outline

Background
Certain viruses contribute to the development of ~15% of human cancers. Preventing or treating these infections therefore provides an opportunity to reduce the human cancer burden globally. However, in order to do so, we need to understand the biology of these oncogenic viruses. Here, we propose to study the most recently identified human tumour virus with a view to developing strategies to prevent its oncogenicity.

The virus is Merkel cell polyomavirus (MCPyV). Infection with MCPyV is associated with the development of Merkel cell carcinoma (MCC), a rare but highly aggressive skin cancer (1). It has now been confirmed that MCPyV is present in approximately 80% of MCC tumours (2).

Polyomaviruses are small, non-enveloped viruses that contain a circular double-stranded DNA genome of approximately 5000 base pairs. The genome contains early and late coding regions, separated by a non-coding regulatory region (NCRR) which contains the viral origin of replication (Ori) and transcriptional regulatory elements (2). Very little is known about the MCPyV life cycle but it thought to be broadly similar to the life cycle of other polyomaviruses. The MCPyV early region encodes a transcript that can be alternatively spliced to encode large T antigen (LT), small T antigen (sT) and a 57 kDa protein (57 kT) that has the same N-terminus as LT, but lacks the C-terminal region of LT. LT is a helicase required for replication of the MCPyV genome and is also thought to bind and degrade the host cell tumour suppressor proteins p53 and pRb. sT has been shown to act as an accessory replication protein, that greatly increases the efficiency of LT-dependent MCPyV replication and transforms cells by inactivating the Akt-mTOR pathway. It has been demonstrated that sT and the N-terminal portion of LT are expressed in tumour samples, suggesting they may together play a role in tumourigenesis. The function of the 57 kT antigen is unknown.

The late region of the MCPyV genome encodes the structural proteins VP1, VP2 and VP3 that assemble to form the virus capsid. Activation of the late region is facilitated by expression of LT leading to a switch in early-to-late gene expression, although the
mechanism of this switch is poorly understood.

Recent work has shown that the cellular protein CCCTC-binding factor (CTCF) is important in the regulation of gene expression in other DNA viruses including Kaposi’s sarcoma-associated herpesvirus (KSHV) and Epstein-Barr virus (EBV). CTCF associates with the genomes of these viruses, regulating the expression of lytic and latent genes by the control of epigenetic markers surrounding key promoter regions (3,4). The known cellular functions of CTCF include transcription insulation, epigenetic boundary formation, genetic imprinting, and the control of mRNA splicing by directing the inclusion of weak exons (5,6).

Bioinformatic analysis of the MCPyV genome suggests it contains three CTCF binding sites. These putative binding sites are interesting for two specific reasons. First, two of the binding sites flank a region of the T antigen transcript that is spliced out to produce the 57 kT protein. Studies have shown that CTCF recruitment to specific regions of the host cell DNA causes pausing of RNA polymerase II, which enhances the inclusion of weak upstream exons. Whether CTCF association with the MCPyV genome is important in regulating the relative expression levels of sT, LT and 57 kT is an interesting question. Second, another putative CTCF binding site has been identified that sits between the transcriptional termination sites of the T antigen early region and the VP1 late gene region. By binding to specific regions within the host cell DNA, CTCF can regulate transcriptional termination and creates physical boundaries within specific regions of DNA to control gene expression. We hypothesise that CTCF is recruited to this site within the MCPyV genome to prevent transcriptional read-through at the early-late gene junction.

Specific aims

1. Characterisation of CTCF binding sites within the MCPyV genome
   Using an in vitro assay already established in our laboratory, the binding of CTCF to the putative binding sites discovered by bioinformatic analysis will be tested. 200 base pair regions of the MCPyV genome containing the putative binding sites will be amplified by PCR and the products labelled with a fluorescent tag (FAM). The MCPyV genome fragments will then be incubated with in vitro translated CTCF protein and binding sites confirmed by electrophoretic mobility shift assay (EMSA).

   Once the binding sites have been confirmed, mutations that prevent CTCF binding will be introduced in the whole MCPyV genome by site-directed mutagenesis. These mutations will be carefully designed such that the amino acid sequence of the proteins encoded at these regions is not altered. The association of CTCF to the mutated binding sites will then be tested using the in vitro binding assay described above.

2. Establishment of a MCPyV culture system and characterisation of CTCF function in the virus life cycle.
   It has been shown that MCPyV can replicate and express the full repertoire of viral genes in the human embryonic kidney cell line 293 (7). In order to establish this assay in our laboratory we will transfect 293 cells with an MCPyV genome clone and study viral replication and maintenance by southern blot analysis. Viral gene expression will also be assessed by real time PCR. Alongside these studies the ability of MCPyV genomes containing mutations in one or more of the CTCF binding sites to replicate and appropriately express all the viral genes will be assessed.

   Studying the MCPyV life cycle in cultured cells, combined with manipulation of the
viral genomes to prevent CTCF association, will provide important insight into the life cycle of this understudied oncogenic human virus and allow us to determine CTCF function in completion of the virus life cycle.

References


How are you planning to ensure adequate supervision?

The student will meet with the supervisors regularly to discuss progress and short and long-term plans. In addition, the student will attend and present at weekly lab meetings. Each week a member of the group presents their most recent experimental data, which is then then discussed in an informal and supportive manner. We also have frequent journal club style meetings in which students and postdocs present a recent publication in our area of interest. These meetings provide useful discussion points and an opportunity for feedback on presentation and critical analysis skills.

In addition, the student will be informally supervised within the laboratory by an experienced postdoctoral research assistant. This provides a firm support network for students in the laboratory that can be adapted to an individual’s specific needs.

The student role.

The student will be trained in all necessary techniques and will therefore perform the laboratory work required to deliver the project goals. Importantly, to ensure ownership of the project, the student will be given every opportunity to contribute intellectually to the direction of the project, through discussion with the supervisors during weekly review meetings and during laboratory group meetings. To aid this intellectual input and to facilitate writing the thesis, the student will be expected to review the literature underpinning the project and to keep abreast of the current literature enabling the development and direction of the project and ensuring it remains cutting edge.
Malignant gliomas represent some of the most aggressive forms of brain cancers (Wen 2008). Despite the most advanced treatment, with combinations of surgery, radiotherapy and chemotherapy, as well as novel molecularly targeted therapies, glioma mortality and morbidity remain very high, with a 5 year survival rate of approximately 3%. Human gliomas express a number of receptor tyrosine kinases (RTKs), including PDGFR and EGFR, and corresponding ligands that contribute to their malignancy by enhancing glioma cell survival, proliferation and migration. Despite the importance of RTK signalling for disease formation and progression, the clinical success of RTK-targeted therapies in gliomas has been modest. This is presumably due to the fact that gliomas are extremely complex and heterogeneous, with many signalling pathways affected. To achieve greater therapeutic efficiency from RTK targeting, it will be important to better understand the molecular determinants of RTK-pathway dependency. To enhance the efficacy of RTK inhibitors, alone or in combination with traditional cytotoxic therapies, it will be vital to identify the factors that determine sensitivity and dependence to RTKs.

Changes in endocytic trafficking plays an active role in the regulation of RTK signalling during cancer progression (Bache 2004). Activated RTKs are internalized and transported through endosomes towards lysosomal degradation which terminates the signals. During this process, receptors can be sorted into recycling endosomes and return to the cell surface to continue signalling. The internalized receptors are associated with many of their downstream effectors, which provide both compartmentalization and temporal regulation of the assembled signalling complexes (Miaczynska 2010). Great progress has been made in describing the endocytic routes, but we are far from understanding how endocytosis and sorting of activated RTKs are regulated. However, oncogene-induced increase in RTK recycling has been associated with tumour growth and metastasis (Muller 2009), highlighting the functional importance of receptor trafficking.

In addition to aberrant RTK activation, mutations in the PI 3-kinase/PTEN pathways are commonly observed in human gliomas. Loss of PTEN increases RTK-induced malignancy, while reducing the success of RTK-targeted experimental and clinical
therapies (Abounader 2009) through poorly understood molecular mechanisms. We recently observed that increased PI 3-kinase signalling enhanced EGFR- and PDGFR activation by promoting receptor internalization through macropinocytosis and subsequent receptor recycling (Schmees et al., 2012, Fig 1). This opens up the possibility that mutations in the PI 3-kinase pathway may enhance glioma cell proliferation by altering the RTK endocytosis route resulting in increased RTK signalling.

Figure 1. Increased PI 3-kinase activity favours RTK internalization via macropinocytosis over clathrin-mediated endocytosis. Macropinocytosis directly causes increased and prolonged RTK activation, accompanied by recruitment of a specific subset of signalling molecules resulting in increased survival signals.

The aim of this project is to investigate how the status of the PI 3-kinase pathway affects EGFR and PDGFR activation and signal transduction. Human glioma cells with wt PI 3-kinase pathway will be compared to glioma cells with inactive PTEN, or harbouring activating PI 3-kinase mutations. These cells will be stimulated with EGF and/or PDGF, and the activation of EGFR, PDGFR and key downstream signalling pathways will be determined using immunoprecipitation and western blot analysis. The importance of the PI 3-kinase pathway status for PDGF and/or EGF-induced proliferation will be assessed using MTT- and soft agar assays. To determine if the PI 3-kinase/PTEN status affect receptor endocytosis, glioma cells will be fixed and stained for RTKs and endosomal markers. Using low molecular weight inhibitors and siRNA, receptor endocytosis will be perturbed to investigate the importance of entry route for RTK signalling.

References:

How are you planning to ensure adequate supervision?

The student will be working on the 5th floor of Biosciences which hosts the Hellberg and Heath labs. Initially Dr. Hellberg will be training and supervising the student directly, but after s/he becomes familiar with the techniques the postdoc and PhD students will help with the daily supervision. The student will participate in data discussions with Dr. Hellberg weekly, and Professor Heath on a monthly basis.

The student role.

The student will be expected to run the project under close supervision by Dr. Hellberg. S/he will learn to independently maintain human glioma cell lines, and to plan and perform state-of-the art biochemical and cell biological assays used to investigate RTK signal transduction. S/he will learn to fix and stain glioma cells for immunofluorescence analysis, and will be trained to use confocal microscopy under supervision.
**Project Title:** Systematic reviews of treatment strategies for germ cell tumours in children and young adults

**Department:** Cancer Research UK Clinical Trials Unit (CRCTU), School of Cancer Sciences

**Discipline:**
- Cancer Sciences
- Pathology
- Metabolic Medicine
- Haematology
- Infection
- Immunology
- Anatomy
- Endocrinology
- Liver & GI Medicine
- Evolutionary basis of clinical medicine

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**Project Outline**

Many paediatric cancers are rare. It has been thought in some quarters to be difficult to perform randomised trials in rare diseases, therefore much of the evidence base for the current standard treatments for germ cell tumours comes from small non-randomised studies, the quality and validity of which may be poor.

Intracranial germ cell tumours (GCTs) are rare tumours of childhood and adolescence, accounting for 3.4% to all malignant tumors in children. The estimated incidence is 0.6 per 100,000 children up to 15 years. They are heterogeneous with respect to their primary site, histology, biological profile and response to treatment. Although co-operative studies have been undertaken, there remains uncertainty as to the best therapeutic strategies. The overall prognosis of paediatric malignant GCTs has increased from around 25% to more than 80%. The current aim of treatment is to reduce the long term effects of therapy in localised GCT and for high risk patients to intensify treatment to improve survival.

The aim of this project is: to perform a systematic literature search to identify reports of therapeutic interventions for intracranial GCTs; to extract information on the treatments evaluated, the study designs, the results and conclusions, and the methodological quality of the studies; and to synthesise this information in systematic reviews, possibly with quantitative meta-analysis if any of the studies address the same therapeutic question and are of sufficient quality. These systematic reviews will help inform clinical practice and contribute to defining the pertinent clinical research questions to be addressed in future trials in order to improve the treatment for these diseases.

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**How are you planning to ensure adequate supervision?**

Keith Wheatley is Professor of Medical Statistics at CRCTU and statistical lead for the Children’s Cancer Trials Team (CCTT) and Jayne Wilson is a Senior Systematic Reviewer at CRCT. We both have substantial experience and expertise in evidence synthesis that will enable us to supervise and educate the student(s) appropriately.
The student role.

The student will learn through working on their systematic review and participation in meetings to discuss the review. Skills that will be acquired will include: designing and conducting a systematic review including writing a protocol; systematically searching for studies using electronic data bases, storing and sorting the results of these searches using databases and reference manager software, data extraction of information and quality assessment of research methods; synthesising data using statistical techniques such as meta-analysis; writing up a scientifically sound but also user friendly report of findings.

Project Title: Systematic reviews of treatment strategies for high grade glioma in children and young adults

Department: Cancer Research UK Clinical Trials Unit (CRCTU), School of Cancer Sciences

Discipline: | Cancer Sciences | Immunology |
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### Project Outline

Many paediatric cancers are rare, including several types of brain tumour such as glioma. Brain tumours in children differ from those in adults in several aspects, including location, histologic diversity, and behaviour. Gliomas are the most common type of brain tumour in childhood, representing more than 50% of the total. Unlike in adults, the majority of gliomas in children are low grade but the high-grade gliomas are a significant clinical challenge. They account for 15% to 20% of childhood gliomas and are characterised by their invasive, aggressive behaviour and unfavourable prognosis. Less than 20% of children diagnosed with high-grade glioma survive 3 years or more.

In general, surgery is performed to relieve symptoms and reduce the tumour, although diffuse intrinsic brainstem tumours are usually considered unresectable and diagnosed based on radiological imaging. Radiotherapy has a role in treatment and is administered focally after maximum surgical resection; however it is avoided in very young child due to the significant adverse effect on brain development. Adjuvant chemotherapy is sometimes tried but the role of chemotherapy continues to be a source of debate. New, effective therapeutic approaches are required for this devastating disease.

It has been thought in some quarters to be difficult to perform randomised trials in rare diseases, therefore much of the evidence base for the current standard treatments for high grade glioma comes from small non-randomised studies, the quality and validity of which may be poor.

The aim of this project is: to perform a systematic literature search to identify report of therapeutic interventions for high grade glioma; to extract information on the treatments evaluated, the study designs, the results and conclusions, and the methodological quality of the studies; and to synthesise this information in systematic reviews, possibly with quantitative meta-analysis if any of the studies address the same therapeutic question and are of sufficient quality. These systematic reviews will help inform clinical practice and contribute to defining the pertinent clinical research questions to be addressed in future trials in order to improve the treatment for these diseases.
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<td>The student will learn through working on their systematic review and participation in meetings to discuss the review. Skills that will be acquired will include: designing and conducting a systematic review including writing a protocol; systematically searching for studies using electronic databases, storing and sorting the results of these searches using databases and reference manager software, data extraction of information and quality assessment of research methods; synthesising data using statistical techniques such as meta-analysis; writing up a scientifically sound but also user friendly report of findings.</td>
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An emerging role for B cells in the pathogenesis of autoimmune liver diseases (AILD) (autoimmune hepatitis/AIH, primary biliary cirrhosis and primary sclerosing cholangitis) has been reported [1, 2]. AILD are characterized by the presence of B cells and plasma cells in the liver and elevated levels of IgG and IgM and the presence of autoantibodies (all of which are secreted by memory B cells) in the blood [3]. B cell subsets include effector B cells, which secrete IFN-gamma, and IL-10 secreting B regulatory cells (B<sub>regs</sub>) which have an anti-inflammatory role in suppressing immune responses. The balance of these effector and regulatory subsets may determine progression and outcome of disease. [4, 5]. In some patients with AIH treatment with Rituximab, an antibody that depletes memory B cells, leads to remission suggesting a significant role for B cells[6]. We have recently shown that the balance between regulatory T cells and effector Th1 and Th17 cells is crucial in determining outcome in autoimmune hepatitis[7, 8] and we would now like to explore the pathogenic role of B cells in AILD. Our preliminary data show there are increased numbers of B cells with high expression of CD39, CD40together with B<sub>regs</sub> in autoimmune liver diseases. In the present project we will determine the function of these B<sub>regs</sub> to show how they control effector responses in autoimmune liver diseases.

**Aim of the project**

To investigate the phenotypic and functional role of B<sub>regs</sub> in human AILD.

**Project outline**

1) Immunohistochemistry of human liver tissue and flow cytometry of cells isolated from blood and liver tissue removed at transplantation will be used to determine the phenotype, frequencies and tissue localisation of B<sub>regs</sub> and B effector subsets in AILD. Secretion of immunoglobulins will be measured and in all studies disease samples will be compared with healthy control blood samples and non-diseased liver tissue from organ donor. All of these techniques and tissues are available in our laboratory.

2) The cytokine secretion of the different liver infiltrating B effector and B regulatory cells will be determined by intracellular cytokine staining.
3) The ability of Bregs to suppress immune responses will be studied in vitro by suppression assay.

4) Finally we will study the effect of the liver microenvironment on the survival, activation and function of B cells by co-culturing B cells with primary human liver cells types including hepatocytes and biliary epithelial cells to determine how paracrine interactions within the inflamed liver microenvironment might affect B cells function in situ.

Benefits: This project will allow the student to learn a range of laboratory techniques and to generate important new data about the pathogenesis of autoimmune liver diseases, a leading cause of liver failure, liver cancer and liver transplantation in UK. The results may suggest novel therapies aimed at B-lymphocytes in the treatment of autoimmune liver disease.

Successful student will have an opportunity to apply for FALK bursary by end of February.

Techniques to be used in the project: Human cell culture and isolation, cells isolation from human peripheral blood B cells subsets from liver clinics and explanted liver tissue; phenotyping with flow cytometry, immunocytochemistry, confocal microscopy, qPCR, functional assays (co-culture, blocking assays, proliferation and survival assays).

How are you planning to ensure adequate supervision?
We have chosen to combine the technical expertise of two supervisors to ensure the student is exposed to the maximal number of transferable research skills. Dr Ye Htun Oo is dedicated to 80% of his time in the laboratory, which is well equipped and set up for this type of study. David Adams is internationally renowned for his work in hepatic immunology and has a proven track record in supervision of both undergraduate and postgraduate students. The Centre for Liver Research is part of the MRC Centre for Immune regulation and includes a large number of scientists working on liver immunology so the student will be working in a stimulating and supportive environment providing the student with exposure to research staff at all stages of their research careers. Appropriate training in research techniques and data analysis and presentation will be provided. We have weekly meetings with supervisors and interactions with other members of the research groups, who will be available day to day for advice and tuition.

The student role.
The student will be expected to perform experiments and conduct data analysis. They will present data to the supervisors and team and wider research groups and be expected to assimilate available published literature under guidance from the supervisors. They will be trained and expected to work using good laboratory practice under local standard operating procedures and be mindful of ethical use of human tissue specimens. They will be working in a large research teams and thus would be expected to interact with colleagues and use local facilities in a respectful manner.

They could have an opportunity to sit in and observe in dedicated autoimmune hepatitis clinics to correlate laboratory bench work findings to pathogenesis and investigation of hepatic inflammation and autoimmunity. This would provide the student with an opportunity to link basic science to the patient.
**Lead Supervisor:** Professor A.M.R. Taylor

**Co Supervisor**
Dr P.J. Byrd

**Project Title:**
Homologous recombination repair deficiency, PALB2 mutation and lymphoid tumours

**Department:**
Cancer Sciences

**Contact Email:**
A.M.R.Taylor@bham.ac.uk

**Telephone:**
Internal Tel: 44488

**Is the project cancer related?**
Yes

**Is the project cancer related?**
Yes

**Discipline:**
- Cancer Sciences
- Immunology
- Pathology
- Anatomy
- Metabolic Medicine
- Endocrinology
- Haematology
- Liver & GI Medicine
- Infection
- Evolutionary biology in Clinical medicine

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**Project Outline**

**Background**
PALB2 is a WD40 repeat domain containing protein, originally identified as a BRCA2 binding protein (Xia et al, 2006). Subsequently it has been demonstrated to function as a molecular bridge that promotes the recruitment of both BRCA1 and BRCA2 to sites of DNA damage by its simultaneous interaction with both proteins via its N-terminal coiled-coil domain and C-terminal WD repeats respectively.

Recently we have identified a non-consanguineous family with two affected sisters who both exhibit mild learning difficulties and mild dysmorphic facial features. Remarkably, both sisters developed B cell non-Hodgkins lymphoma at ages 10 and 12 years. We identified biallelic PALB2 mutations by total exomic sequencing and therefore, this is the first observation suggesting a role for PALB2 in the development of lymphoma. Western blotting showed absence of expression of full length PALB2 together with a slightly smaller and unstable PALB2 protein. From a consideration of the PALB2 mutations present, PALB2 coiled coil N-terminal sequence (aa9-44), that interacts with BRCA1 (Fan Zhang et al, 2009; Feng Zhang et al, 2009) is, therefore, assumed to be present for both alleles, whereas the C-terminal PALB2 WD-40 repeats that associate with BRCA2 are absent in one allele but present in the other as is the MRG15 binding domain. Cells from these individuals showed no Rad51 foci. The normal appearance of γ-ray induced BRCA1 foci in these sister’s cells is consistent with BRCA1 acting upstream of PALB2 (Fan Zhang et al, 2009). PALB2 links BRCA1 to BRCA2 with the order of binding events believed to be BRCA1>PALB2>BRCA2 which then regulates assembly of Rad51 (Fan Zhang et al, 2009; Feng Zhang et al, 2009).

Whether there is residual PALB2 function in these sisters’ cells is, as yet, unknown but it is clear that the clinical phenotype appears to be milder and results in a different tumour specificity to that previously described.

**References**


**The student role.**

The student would investigate the function of the mutant PALB2 expressed by the sisters described above in two ways (i) by PALB2 co-immunoprecipitation experiments using extracts from the patients’ cells and (ii) by expression of the mutant proteins in an isogenic system that would allow comparisons of wild type protein functions with mutant protein functions. Co-immunoprecipitation experiments would determine whether the mutant proteins retain any ability to interact with BRCA1 and BRCA2. This is important because PALB2 has no intrinsic enzymatic activity and its principle role and function may be through its ability to bind BRCA1 and BRCA2, leading to recruitment of Rad51 at sites of DNA damage. The isogenic expression system is a U2OS cell based Flp-In/T-Rex system. This system allows doxycycline induced FLAG-tagged mutant PALB2 protein to be expressed in cells in which the endogenous wild type protein has been depleted using siRNA. The functions of wild type and mutant PALB2 protein can then be compared in the same cellular background. Using this system and co-immunoprecipitation of mutant PALB2 from the patients’ cells, experiments will be carried out to investigate the function of the PALB2 protein in the response to DNA damage.
Project Outline

Relapse rates for AML are dismal ranging from 40% in children to over 70% in adults over 60 years.\(^1\)\(^-\)\(^3\) Despite this, morphological first remission is induced in the majority of patients which suggests a role for chemo-resistant leukaemia stem cells (LSCs) in the generation of relapsed disease. Heterogeneity in AML LSCs has recently become evident and the dogma that AML LSCs are only CD34+ is no longer substantiated. Using more permissive animal models, LSC activity is evident in CD34-CD38- fractions of AML.\(^4\)\(^-\)\(^6\) In normal haemopoiesis, these cells represent a poorly studied population with long term repopulating activity, which appear to be more primitive than the classical CD34+ haemopoietic stem cell.\(^7\)\(^,\)\(^8\) As LSCs are detectable in multiple cell fractions from many AMLs, the identity of those LSCs responsible for relapse are not clear.\(^5\)\(^,\)\(^6\) Insight into differences in chemo-resistance of different LSCs is required for their characterisation and could reveal useful new therapeutic targets.

We have recently addressed chemo-resistance of different AML subpopulations and found that, in a particular group of childhood AMLs with a striking tendency for reduced relapse-free survival, the CD34-CD38- cells are most resistant to chemotherapy \textit{in vitro} as well as \textit{in vivo} using colony forming cell assays and murine engraftment models.\(^9\) Global gene expression profiling of these cells revealed elevated expression of stress responses genes such as heat shock protein and glutathione redox genes and an undifferentiated phenotype. Importantly, serial analysis revealed that not all of these LSCs are equally able to resist chemotherapy following prolonged exposure. By interrogating a subgroup of AMLs enriched for highly chemo-resistant LSCs, we were able to derive an underlying network of pathways which include specific cell surface markers.

In the first part of the study, the student will validate the ability of the identified unique cell surface markers to identify chemo-resistant CD34-CD38- LSCs. We have identified at least 7 candidate genes which encode transmembrane/cell surface proteins which, in combination, could provide novel identifiers for pure chemo-resistant LSCs. Commercial antibodies are available for all proteins. The student will use flow cytometry to demonstrate that these cell surface markers validate our microarray data. They will then use them to isolate chemo-resistant LSCs and
functionally validate their chemo-resistance and stem cell activity though serial colony forming cell assays in the presence of chemotherapy. Isolated chemo-resistant colonies can be tested for the expression of other genes identified in our network analysis. The identification of unique cell surface markers for chemo-resistant LSCs could have major implications for the diagnosis and management of AML. In the second part of the study, the student will evaluate the impact of silencing specific genes that are unique to the chemo-resistant LSCs on their function. The student will use lentivirus mediated-short hairpin RNA to silence expression of highly expressed cell surface markers and/or additional validated targets to determine the effect on chemo-resistance and/or leukaemia propagating activity using serial colony forming cell assays and a murine engraftment model. Should knockdown have an effect on LSC function, these could provide novel therapeutic targets for chemo-resistant LSCs in high-risk AML.


**How are you planning to ensure adequate supervision?**

Dr Weston will be available for day to day supervision in the laboratory. Once both supervisor and student are happy that the student is proficient in the required techniques (flow cytometry, qPCR, colony forming cell assays and lentiviral-mediated gene silencing) they will be left to work independently and seek guidance when required. The group has a technician who performs animal work. However, the student will have the opportunity to obtain their home office animal handling licence should they wish. While Dr Weston and the student will interact on a daily basis, progress will be monitored thoroughly through more formal weekly and monthly meetings.

**The student role.**

The student will be expected to manage their time and work independently. They should communicate problems and seek guidance when necessary without hesitation and will be encouraged to problem solve following discussion. They will interpret data and communicate their findings.
Lead Supervisor: Dr Ye Htun Oo
Co Supervisor: Prof David Adams

Project Title: Investigating of role of intrahepatic regulatory T cells and dendritic cells interaction in hepatic inflammation.
Department: Centre for Liver Research & NIHR BRU, 5th Floor, IBR. UHB NHS Foundation Trust
Contact Email: Telephone: y.h.oo@bham.ac.uk d.h.adams@bham.ac.uk
Is the project cancer related? No
Discipline: Cancer Sciences Anatom Immunology
Pathology Endocrinology
Metabolic Medicine Liver & GI Medicine
Haematology Evolutionary biology in Clinical medicine

Project Outline
Regulatory T cells (T\textsubscript{regs}) are crucial in maintaining hepatic tolerance and preventing autoimmunity[1-3]. Dendritic cells (DC) are antigen-presenting cells and they are important modifier of T\textsubscript{regs}. T\textsubscript{regs} and DC reside closely in inflamed human liver. DC express MHC class 2, co-stimulatory molecules and CD70 and toll like receptors that sense pathogen and intrinsic danger signals. They produce cytokines IL-1, IL-6, IL-10 and TGF-beta. We have shown that presence of DC in the inflamed human liver.

Our preliminary data shows that CD27 (which interact with CD70) is highly expressed on liver infiltrating T\textsubscript{regs}[2] but very little is known about role of DC regulating T\textsubscript{regs} in hepatic inflammation. Thus now we like to elucidate the detailed phenotype and functional cellular regulation role of dendritic cells and T\textsubscript{regs} in inflamed human liver.

Experiment plan
1. Investigate the localization of DC and relation to Tregs and other immune cells in hepatic inflammation with double immunohistochemistry and confocal microscopy.
2. Phenotype the surface markers and intracellular cytokines expression of DC from explant liver tissue from transplant patients.
3. To investigate whether soluble (IL-6) or contact dependent (CD70-CD27) mechanism of DC regulation of intrahepatic T\textsubscript{regs} and/or effector CD4 and CD8 cells apoptosis or proliferation with cell to cell/ cell in soluble media co-culture and blocking experiments.
4. To examine the anti-fibrotic potential of DC by co-culturing with fibroblasts and neutralizing anti-TGFbeta.

**Benefits:** This project will allow the student to learn a range of laboratory techniques and to generate important new data about the pathogenesis of autoimmune liver diseases, a leading cause of liver failure, liver cancer and liver transplantation in UK. The results may suggest novel therapies aimed at B-lymphocytes in the treatment of autoimmune liver disease.

**Successful student will have an opportunity to apply for FALK bursary by end of February.**

**Techniques to be used in the project:** Expanted human liver tissue from transplant programme will be used. Human cell culture and isolation, cells isolation from human peripheral blood and lymphocyte subsets from liver clinics and explanted liver tissue; phenotyping with flow cytometry, immunocytochemistry, confocal microscopy, qPCR, functional assays (co-culture, blocking assays, proliferation and survival assays).

**How are you planning to ensure adequate supervision?**

We have chosen to combine the technical expertise of two supervisors to ensure the student is exposed to the maximal number of transferable research skills. Dr Ye Htun Oo is dedicated to 80% of his time in the laboratory, which is well equipped and set up for this type of study. David Adams is internationally renowned for his work in hepatic immunology and has a proven track record in supervision of both undergraduate and postgraduate students. The Centre for Liver Research is part of the MRC Centre for Immune regulation and includes a large number of scientists working on liver immunology so the student will be working in a stimulating and supportive environment providing the student with exposure to research staff at all stages of their research careers. Appropriate training in research techniques and data analysis and presentation will be provided. We have weekly meetings with supervisors and interactions with other members of the research groups, who will be available day to day for advice and tuition.

**The student role.**

The student will be expected to perform experiments and conduct data analysis. They will present data to the supervisors and team and wider research groups and be expected to assimilate available published literature under guidance from supervisors. They will be trained and expected to work using good laboratory practice under local standard operating procedures and be mindful of ethical use of human tissue specimens. They will be working in large research teams and thus would be expected to interact with colleagues and use local facilities in a respectful manner.

They could have an opportunity to sit in and observe in dedicated autoimmune hepatitis clinics to correlate laboratory bench work findings to pathogenesis and investigation of hepatic inflammation and autoimmunity. This would provide the student with great opportunity to link basic science to patient.
**Lead Supervisor:** Dr Ye Htun Oo

**Co Supervisor**
Prof David Mutimer

**Project Title:** Investigating of functional role of NK cells in Hepatitis C recurrence and cirrhosis in liver transplant patients

**Department:** Centre for Liver Research & NIHR BRU, 5th Floor, IBR. UHB NHS Foundation Trust

**Contact Email:** y.h.oo@bham.ac.uk
david.mutimer@uhb.nhs.uk

**Is the project cancer related?** No

**Discipline:**
- Cancer Sciences
- Pathology
- Metabolic Medicine
- Haematology
- Infection
- Immunology
- Anatomy
- Endocrinology
- Liver & GI Medicine
- Evolutionary biology in Clinical medicine

**Project Outline**
Hepatitis C virus (HCV) induced liver injury is the primary indication for liver transplantation (LT) in United Kingdom. Recurrent HCV infection occurs in universal, and nearly 20% of these patients go on to develop fibrosis and cirrhosis. HCV virus is detectable in the blood within 12 h of reperfusion of the allograft and generally returns to pre-transplant baseline levels within days of transplantation[1]. LT recipients with HCV infection have higher rates of transplanted liver cirrhosis and death compared to virus-free recipients[2, 3].

The liver lymphocyte population is enriched with natural killer (NK) cells, which play a key role in host defense against viral infection. Recent animal models suggests that NK cells play an important role in inhibiting liver fibrosis by selectively killing hepatic stellate cells (HSCs) and also by producing the anti-fibrotic cytokine IFN-γ [4], on the other hand chemokine CXCL10 has been shown to promote liver fibrosis[5]. Our preliminary data suggested that NK cells frequency in HCV post-LT patients are lower if they have graft cirrhosis and HCV liver has high level of CXCL10. Thus now we like to elucidate the detailed phenotype and functional role of NK cells. We also like to characterize NK cells’ regulation of hepatic stellate cells.

**Aim of the project**
Investigating of functional role of NK cells in Hepatitis C recurrence and cirrhosis in liver transplant patients.

**Project outline**
1. Investigating the phenotype of NK cells from peripheral blood of post transplant HCV graft hepatitis and cirrhosis patients (Genotype 1 vs non-Genotype 1) and assess profibrotic chemokines (e.g. IP-10) and cytokines (IL4, IL5, IL33) compared with those who does not have graft fibrosis or alcoholic liver transplant patients.
2. Flow cytometry to phenotype the surface characteristic of liver infiltrating NK cells from explant liver from HCV- transplant patients (Genotype 1 vs non-Genotype 1).
3. Purified NK cells from post LT untreated HCV RNA+patients, pegylated interferon-treated patients and healthy controls to co-cultured with activated primary HSCs from explanted liver, and were tested for hepatic stellate cell...
apoptosis.

4. To investigate whether soluble or contact dependent mechanism was involved in HSC apoptosis was contact dependent by blocking antibodies specific for TRAIL, NKG2D and FasL.

5. To examine the anti-fibrotic potential of a neutralizing anti-CXCL10 antibody.


**Benefits:** This project will allow the student to learn a range of laboratory techniques and to generate important new data about the pathogenesis of fibrosis in HCV transplant patients. Liver diseases, a leading cause of liver failure, liver cancer and liver transplantation in UK. The results may suggest novel therapies aimed at NK-lymphocytes in the treatment of HCV related liver fibrosis.

**Techniques to be used in the project:** Human cell culture and isolation, cells isolation from human peripheral blood from liver clinics and explanted liver tissue; phenotyping with flow cytometry, immunocytochemistry, confocal microscopy, qPCR, functional assays (co-culture, blocking assays, proliferation and survival assays).

**How are you planning to ensure adequate supervision?**

We have chosen to combine the technical expertise of two supervisors to ensure the student is exposed to the maximal number of transferable research skills. Currently, Dr Ye Htau Oo’s group has a post-doctoral scientist, one student, one clinical fellow and a senior technician. Dr Oo is dedicated to 80% of his time in the liver laboratory research so proper research team will supervise the student. Professor David Mutimer is internationally renowned for his expertise and knowledge in chronic viral hepatitis and has wealth of experience in clinical hepatology. He is the lead clinician for viral hepatitis and runs clinical trials with new antivirals medication. All supervisors have a proven track record in supervision of both undergraduate and postgraduate students and work in a large research group providing the student with exposure to research staff at all stages of their research careers. Appropriate training in research techniques and data analysis and presentation will be supplied. We will have weekly meetings with supervisors and interactions with other members of the research groups, who will be available day to day for advice and tuition.
The student role.

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Liver disease is the 5th most common cause of death in the UK. Clinical observations have suggested that the number of cases of hepatocellular carcinoma (HCC) is increasing. Cell death is common in HCC and this leads to release of adenosine triphosphate (ATP). ATP binds to its receptor on hepatocyte and plays an important role in mediate cellular proliferation and regeneration [1, 2]. Expression of this receptor has been linked to the pathogenesis of liver cancer in murine model and also in colonic cancer[3, 4].

ATP is transformed to AMP by CD39 and subsequently to Adenosine by CD73. We have recently demonstrated that CD39 is highly expressed on intrahepatic regulatory T cells especially in inflamed human liver[5, 6]. We now like to investigate the hepatic up-regulation of the ATP receptor in endothelial cells of hepatocellular carcinoma and CD39 and CD73 expression in tumor infiltrating lymphocytes. We know hope to elucidate whether generating adenosine by CD39 and CD73 on T cells and hepatocellular carcinoma tumor endothelium would prevent T effector cell proliferation and foster angiogenesis for growth of liver cancer. If we could investigate the mechanism, blocking this pathway may be effective therapy in HCC.

Benefits: This project will allow the student to learn a range of laboratory techniques and to generate important new data about the pathogenesis of liver cancer, a leading cause of liver transplantation in UK. The results may suggest novel therapies aimed at lymphocytes in the treatment of liver cancer.

Techniques to be used in the project: Primary human cells from our transplant programme will be used. Human cell culture and isolation, confocal microscopy, qPCR, flow cytometry, immunocytochemistry, ELISA, functional assays (survival, apoptosis)
### How are you planning to ensure adequate supervision?

We have chosen to combine the technical expertise of two supervisors to ensure the student is exposed to the maximal number of transferable research skills. Currently, Dr Ye Htun Oo has a post-doctoral scientist and he is dedicated to 80% of his time in the liver laboratory research so proper research team will supervise the student. Dr Tahir Shah, clinical service lead in liver medicine is internationally renowned for his expertise and knowledge in hepatic tumour, neuroendocrine tumour and dedicated liver cancer clinics. He is the lead clinician for hepatocellular cancer clinics. All supervisors have a proven track record in supervision of both undergraduate and postgraduate students and work in a large research group providing the student with exposure to research staff at all stages of their research careers. Appropriate training in research techniques and data analysis and presentation will be supplied. We will have weekly meetings with supervisors and interactions with other members of the research groups, who will be available day to day for advice and tuition.

### The student role.

The student will be expected to perform experiments and conduct data analysis. They will present data to the supervisory team and wider research groups and be expected to assimilate available published literature under guidance from supervisors. They will be trained and expected to work using good laboratory practice under local standard operating procedures and be mindful of ethical use of human tissue specimens. They will be working in large research teams and thus would be expected to interact with colleagues and use local facilities in a respectful manner.

### Key References:


**Lead Supervisor:** Dr Ye Htun Oo (MRC Clinician Scientist and Consultant Hepatologist)

**Co Supervisor** Dr Tariq Iqbal (Reader in Gastroenterology)

**Project Title:** Investigating of role of hepcidin in liver inflammation.

**Department:** Centre for Liver Research & NIHR BRU, 5th Floor, IBR. UHB NHS Foundation Trust

**Contact Email:** y.h.oo@bham.ac.uk
**Telephone:** Tariq.Iqbal@uhb.nhs.uk

**Is the project cancer related?** No

**Discipline:**
- Cancer Sciences
- Pathology
- Metabolic Medicine
- Haematology
- Infection
- Immunology
- Anatomy
- Endocrinology
- Liver & GI Medicine
- Evolutionary biology in
  - Clinical medicine

**Project Outline**

Lymphocytes are main players in hepatic inflammation (1). Hepcidin is a peptide hormone produced by the liver hepatocytes and it is the master regulator of iron homeostasis in humans and other mammals. Hepcidin functions to regulate iron transport across the gut mucosa, thereby preventing excess iron absorption and maintaining normal iron levels within the body (2,4). It is a cationic peptide that has broad antibacterial and antifungal actions and it provides a first line of defence at mucosal barriers. Cholestatic liver disease especially primary sclerosing cholangitis has recurrent biliary infections. Most of the T cells are located around the portal tract where the cholangitis occurs. We have shown that both effector and regulatory T cells are present in inflamed liver (1,3) and important role of hepcidin in liver (2,4).

Now we like to elucidate the role of hepcidin interaction with hepatic lymphocytes in preventing liver inflammation.

**Aim of the project**

To investigate the role of hepcidin in human liver inflammation

**Benefits:** Autoimmune liver diseases are the leading cause of liver failure, liver cancer and liver transplantation in UK. Controlling hepatic inflammation would be the key step to prevent liver cirrhosis and liver cancer in the era of donor shortage. This will be the first study to investigate the role of hepcidin in human liver tissue. We could apply this knowledge in the translational medicine to targeting hepcidin to prevent cirrhosis, liver failure and liver transplantation.

**Techniques to be used in the project:** Human cell culture and isolation, cells isolation from human peripheral blood T cells subsets from liver clinics and explanted liver tissue; phenotyping with flow cytometry, ELISA on hepcidin, immunocytochemistry, confocal microscopy, qPCR, functional assays (co-culture, blocking assays, proliferation and survival assays).
How are you planning to ensure adequate supervision?

We have chosen to combine the technical expertise of two supervisors to ensure the student is exposed to the maximal number of transferable research skills. Currently, Dr Ye Htun Oo has a post-doctoral scientist and he is dedicated to 80% of his time in the liver laboratory research so proper research team will supervise the student. Dr Tariq Iqbal, is internationally renowned for his expertise and knowledge in hepcidin and Iron metabolism. He is the lead clinician for basic research at Gastroenterology unit. All supervisors have a proven track record in supervision of both undergraduate and postgraduate students and work in a large research group providing the student with exposure to research staff at all stages of their research careers. Appropriate training in research techniques and data analysis and presentation will be supplied. We will have weekly meetings with supervisors and interactions with other members of the research groups, who will be available day to day for advice and tuition.

The student role.

The student will be expected to perform experiments and conduct data analysis. They will present data to the supervisors and team and wider research groups and be expected to assimilate available published literature under guidance from supervisors. They will be trained and expected to work using good laboratory practice under local standard operating procedures and be mindful of ethical use of human tissue specimens. They will be working in large research teams and thus would be expected to interact with colleagues and use local facilities in a respectful manner.

They could have an opportunity to sit in and observe in dedicated autoimmune hepatitis clinics to correlate laboratory bench work findings to pathogenesis and investigation of hepatic inflammation and autoimmunity. This would provide the student with great opportunity to link basic science to bed side patient’s care.

An emerging role for B cells in the pathogenesis of autoimmune liver diseases—AILD (autoimmune hepatitis/AIH, primary biliary cirrhosis and primary sclerosing cholangitis) has been reported [1, 2]. AILD are characterized by the presence of B cells and plasma cells in the liver and elevated levels of IgG and IgM and the presence of autoantibodies (all of which are secreted by memory B cells) in the blood [3]. B cell subsets include effector B cells, which secrete IFN-gamma, and IL-10 secreting B regulatory cells (Bregs) which have an anti-inflammatory role in suppressing immune responses. The balance of these effector and regulatory subsets may determine progression and outcome of disease. [4, 5]. In some patients with AIH treatment with Rituximab, an antibody that depletes memory B cells, leads to remission suggesting a significant role for B cells[6]. We have recently shown that the balance between regulatory T cells and effector Th1 and Th17 cells is crucial in determining outcome in autoimmune hepatitis[7, 8] and we would now like to explore the pathogenic role of B cells in AILD. Our preliminary data show there are increased numbers of B cells with high expression of CD39, CD40together with Bregs in autoimmune liver diseases. In the present project we will determine the function of these Bregs to show how they control effector responses in autoimmune liver diseases.

**Aim of the project**

To investigate the phenotypic and functional role of Bregs in human AILD.

**Project outline**

1) Immunohistochemistry of human liver tissue and flow cytometry of cells isolated from blood and liver tissue removed at transplantation will be used to determine the phenotype, frequencies and tissue localisation of Bregs and B effector subsets in AILD. Secretion of immunoglobulins will be measured and in all studies disease samples will be compared with healthy control blood samples and non-diseased liver tissue from organ donor. All of these techniques and tissues are available in our laboratory.

2) The cytokine secretion of the different liver infiltrating B effector and B regulatory cells will be determined by intracellular cytokine staining.
3) The ability of Bregs to suppress immune responses will be studied in vitro by suppression assay.

4) Finally we will study the effect of the liver microenvironment on the survival, activation and function of B cells by co-culturing B cells with primary human liver cells types including hepatocytes and biliary epithelial cells to determine how paracrine interactions within the inflamed liver microenvironment might affect B cells function in situ.

**Benefits:** This project will allow the student to learn a range of laboratory techniques and to generate important new data about the pathogenesis of autoimmune liver diseases, a leading cause of liver failure, liver cancer and liver transplantation in UK. The results may suggest novel therapies aimed at B-lymphocytes in the treatment of autoimmune liver disease.

**Successful student will have an opportunity to apply for FALK bursary by end of February.**

**Techniques to be used in the project:** Human cell culture and isolation, cells isolation from human peripheral blood B cells subsets from liver clinics and explanted liver tissue; phenotyping with flow cytometry, immunocytochemistry, confocal microscopy, qPCR, functional assays (co-culture, blocking assays, proliferation and survival assays).

**How are you planning to ensure adequate supervision?**

We have chosen to combine the technical expertise of two supervisors to ensure the student is exposed to the maximal number of transferable research skills. Dr Ye Htun Oo is dedicated to 80% of his time in the laboratory, which is well equipped and set up for this type of study. David Adams is internationally renowned for his work in hepatic immunology and has a proven track record in supervision of both undergraduate and postgraduate students. The Centre for Liver Research is part of the MRC Centre for Immune regulation and includes a large number of scientists working on liver immunology so the student will be working in a stimulating and supportive environment providing the student with exposure to research staff at all stages of their research careers. Appropriate training in research techniques and data analysis and presentation will be provided. We have weekly meetings with supervisors and interactions with other members of the research groups, who will be available day to day for advice and tuition.

**The student role.**

The student will be expected to perform experiments and conduct data analysis. They will present data to the supervisors and team and wider research groups and be expected to assimilate available published literature under guidance from the supervisors. They will be trained and expected to work using good laboratory practice under local standard operating procedures and be mindful of ethical use of human tissue specimens. They will be working in a large research teams and thus would be expected to interact with colleagues and use local facilities in a respectful manner.

They could have an opportunity to sit in and observe in dedicated autoimmune hepatitis clinics to correlate laboratory bench work findings to pathogenesis and investigation of hepatic inflammation and autoimmunity. This would provide the student with an opportunity to link basic science to the patient.
Lead Supervisor: Dr Nils P Krone

Co Supervisor: Dr Ferenc Mueller

Project Title: Novel approaches to explore systemic consequences of disrupted steroidogenesis

Department: School of Clinical and Experimental Medicine

Contact Email: 0121 414 2540, n.p.krone@bham.ac.uk

Telephone: 0121 414 2895, f.mueller@bham.ac.uk

Is the project cancer related? Yes or No

Discipline:
- Cancer Sciences
- Immunology
- Pathology
- Anatomy
- Metabolic Medicine
- Endocrinology
- Haematology
- Liver & GI Medicine
- Infection
- Evolutionary biology in Clinical medicine

Project Outline

Background: Congenital adrenal hyperplasia (CAH) ranks amongst the most common inherited metabolic diseases and comprises a group of inborn errors of steroidogenesis. Over 90% of steroid synthesis is carried out by cytochrome P450 (CYP) enzymes. CYP type 1 are active in the mitochondrion functionally relying on electron transfer from ferredoxin (FDX1) and ferredoxin reductase (FDR). CYP type 2 are localised to the endoplasmic reticulum and depend on P450 oxidoreductase as electron donor. FDX1/ FDR dependent CYP type 1 facilitate over 70% of mineralocorticoid and 50% of glucocorticoid synthesis. Our current work refines steroidogenesis in zebrafish (zf). We showed that zCyp11a2 is the human P450 side-chain cleavage (CYP11A1) ortholog facilitating steroidogenesis from larval stages to adult life rather than the previously published zCyp11a1. Our data highlights the outstanding value of zCyp11a2-deficient zf for modelling impaired steroidogenesis and translational medicine. In addition, we have strong in vivo evidence that Fdx1b interacts as electron donor with zCyp11a2. This conclusively links with our previous findings defining novel forms of CAH due to deficient human CYP11A1. In patients a considerable phenotypic variability was not explained by the genetic defect. Thus, we hypothesise that mitochondrial redox potential regulation is a key modulator in the molecular pathogenesis of CAH. Such mechanisms are likely to have a significant role in systemic health problems; thus models of impaired steroidogenesis can provide novel insights into mechanisms involved in common disease such as hypertension. Thus the main aim of this project is to define in vivo regulation of mitochondrial steroidogenesis as novel regulatory mechanism.

This aim will be addressed by two main Tasks:

1. To develop a TALEN zCyp11a2 null allele strain to study the interrenal and systemic consequences of disrupted steroidogenesis
2. To develop an interrenal (adrenal) specific fluorescent reporter line for in vivo visualisation of interrenal defects and FACS-sorting of interrenal cells.

Task 1: A zCyp11a2 null allele will be created using the TALEN method. Null alleles will be confirmed by RT-PCR with specific primers and in vivo zCyp11a2 deficiency will be confirmed by measuring the interrenal steroid metabolome by liquid
chromatography/tandem mass spectrometry. All these methods are already fully established. Once the strain is available, we will be able to also study the effects of a zCyp11a2 deletion after 5 days post fecundation and in adults, which is not reliably possible following a morpholino approach. More importantly, we will be able to compare the consequences of the zCyp11a2 null allele to a currently developed knock-out of Fdx1b (PhD project Aliesha Griffin). These studies will allow to dissect differential pathways and to distinguish between primary effects due to the gene deletions and secondary general effects due to impaired steroid hormone synthesis. Ultimately, we will use these strains to explore the transcriptomic and steroid metabolomic response during development and adulthood. In addition, we will use these strains to isolate interrenal cells with the help of the reporter strain to be developed as outlined under goal 2.

**Task 2:** We will create a fluorescent interrenal reporter line by cloning the proximal promoter of the interrenal gland specific zHsd3b2 gene into the pDB896-YFP vector. The pDB896:zHsd3b2-YFP construct will be co-injected along with Tol2 mRNA into 1-cell stage embryos to generate the interrenal specific zHsd3b2:YFP reporter line. This line can then be crossed with null alleles of enzymatic (zCyp11a2) and co-factors (zFdx1b) for *in vivo* visualisation of interrenal gland defects to dissect the differential consequences. In addition, the reporter will be employed for FACS-sorting of interrenal cells in transgenic zebrafish allowing for specific isolation of interrenal cells with different steroidogenic defects. These will be used for RNA sequencing to explore the differential transcriptomic responses to specific deficiencies of steroidogenesis during development and in adulthood.

This project will establish two exciting *in vivo* models exploring the interrenal and systemic consequences of disrupted steroidogenesis and provide novel insights into common disease and help developing a completely novel field in endocrinology.

**How are you planning to ensure adequate supervision?**

The proposed project is well embedded into ongoing research employing zebrafish to explore systemic consequences of disrupted steroid hormone synthesis and action. These projects are funded by European charities (IFCAH-ESPE) and the EU *fp7* framework program. Overarching structured supervision will formally take place at least once weekly during lab meetings and on an informal basis when required with the supervisors. To guarantee the maximum scientific outcome (presentations, publications) and the best possible work experience (acquiring scientific skills and broad cutting edge methods) the student will be able to get all required support during daily interaction with postdocs and PhD students working on related projects in the lab.

**The student role.**

Throughout the project, the student will grow into the role with support of our research teams to perform experiments with an increasing level of independence. Together with the student, we will develop experimental outlines to enforce a successful outcome of the proposed studies. Over the course of the project, the student will acquire a multitude of generally applicable lab methods including PCR, cloning, plasmid DNA (MiniPrep, MidiPrep) and RNA preparation, transformation, transfection, cell culture techniques, injection of zebrafish embryos, light and fluorescence microscopy, mRNA synthesis and purification. These methods will equip the student with the required skill mix to gain independence during the daily work. This will also provide the vital basis for a potential career in academic medicine. In addition, the student will acquire basic skill in cutting edge technologies such a steroid metabolome analysis by LC-MS/MS. We see the student as an integral member of our research teams and will provide them with state-of-the-art experience and knowledge to master projects and in translational medicine.
**Lead Supervisor:** Professor Roy Bicknell

**Co Supervisor** Dr Ashley Martin

**Project Title:** Identification of new targets in colon cancer

**Department:** Cancer Sciences

**Contact Email:**
- Bicknell 44085 R.Bicknell@bham.ac.uk
- Martin 49531 A.Martin@bham.ac.uk

**Is the project cancer related?** Yes

**Discipline:**
- Cancer Sciences
- Pathology
- Metabolic Medicine
- Haematology
- Infection
- Immunology
- Anatomy
- Endocrinology
- Liver & GI Medicine
- Evolutionary biology in Clinical medicine

**Project Outline**

There exists an urgent need for new targets in colon cancer. Much cancer research is currently focussed on the tumour vasculature, both in the search for anti-angiogenics and for vascular targeting agents. This project will use a novel proteomic approach to identify new targets lying within the colon tumour vasculature. Professor Bicknell has developed methods that enable the rapid digestion of healthy or cancer colon tissue to single viable cells. The endothelial cells are then captured on labelled magnetic beads and separated from the other cell types.

We propose to surface biotinylate these endothelial cells while they are on the beads using an approach that ensures that only proteins on the outside of the intact cells are labelled. The Proteomic Group, lead by Dr Martin, have used this approach to identify platelet surface proteins previously. Once the cell surface proteins are labelled with biotin proteins will be extracted from the cells and the endothelial surface proteins captured on avidin beads. By analysing these surface proteins from healthy/normal tissue and tumour tissue we will identify and quantify tumour endothelial specific proteins. The relative abundance (quantitation) of each protein identified is made possible by the use of mass spectrometry based proteomic approaches where isotopically labelled reagents are used to differentially mass tag peptides from the two samples. This enables us quantitate several thousand proteins in each analysis.

This cutting edge research uses novel methods, in both the sample generation and analysis, developed in the supervisor’s laboratories. The colon cancer specific endothelium proteins identified have the potential to be medically useful in a variety of ways.

All ethics and logistics required to obtain healthy and tumour colon tissue are in place.
How are you planning to ensure adequate supervision?

Professor Bicknell will provide all of the training and supervision related to the isolation of the control and colon cancer endothelial cells. Professor Bicknell is a leading expert in this field and he, and experienced members of his research group, will provide clear instruction/training for all the procedures.

Dr Martin will provide the training/supervision for the biochemical and proteomic aspects of the work. This will include the preparation of samples for analysis using the proteomic protocols developed in his laboratory in addition to operating the HPLC/Mass spectrometers for analysing the samples. Full training in data mining programs will be provided as will the procedures required for interpretation of the results obtained.

Professor Bicknell and Dr Martin are both experienced at training/supervising students and the complementary experience of the 2 supervisors will ensure that the student receives expert input in all of the techniques utilized. Furthermore, Professor Bicknell has experienced members of his group that will provide “on spot help” as and when required as will members of Dr Martin’s proteomic group.

Both supervisors will have regular contact with the student during the work at the level of day to day supervision and formal meetings will be held every 2-3 weeks to monitor progress. Feedback from the student about the quality of training will be taken at these meetings and any changes required will be carried out.

The student role.

The Student will be trained to isolate the endothelial cells from tissues in order that they can prepare their own samples for the purification of cell surface proteins. The protocols to perform this work are performed routinely in the Bicknell group and training will be given by subject specific experts. Similarly the student will receive a thorough training in the protein biochemistry elements of the work to be able to isolate the surface proteins from the endothelial cells, to generate and isotopically label tryptic peptides. These peptides will be separated by reverse phase HPLC and sprayed on-line directly into an ESI source on a ToF/ToF mass spectrometer. The mass spectrometer will perform an automated MS MS/MS procedure so that the peptides are fragmented and can be identified. The student will be shown how to do this and how to search the data to identify and quantify the proteins and to assess the quality of the data. Therefore, the student will be responsible for all aspects of the work and in doing so will be fully trained in a range of fundamental techniques that will be widely applicable in future work.
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<tr>
<th><strong>Lead Supervisor:</strong></th>
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<tr>
<td><strong>Contact Email:</strong></td>
<td>Bicknell     44085 <a href="mailto:R.Bicknell@bham.ac.uk">R.Bicknell@bham.ac.uk</a></td>
</tr>
<tr>
<td><strong>Telephone:</strong></td>
<td>Martin 49531 <a href="mailto:A.Martin@bham.ac.uk">A.Martin@bham.ac.uk</a></td>
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**Project Outline**

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This cutting edge research uses novel methods, in both the sample generation and analysis, developed in the supervisor’s laboratories. The colon cancer specific endothelium proteins identified have the potential to be medically useful in a variety of ways.

All ethics and logistics required to obtain healthy and tumour colon tissue are in place.
How are you planning to ensure adequate supervision?

Professor Bicknell will provide all of the training and supervision related to the isolation of the control and colon cancer endothelial cells. Professor Bicknell is a leading expert in this field and he, and experienced members of his research group, will provide clear instruction/training for all the procedures. Dr Martin will provide the training/supervision for the biochemical and proteomic aspects of the work. This will include the preparation of samples for analysis using the proteomic protocols developed in his laboratory in addition to operating the HPLC/Mass spectrometers for analysing the samples. Full training in data mining programs will be provided as will the procedures required for interpretation of the results obtained. Professor Bicknell and Dr Martin are both experienced at training/supervising students and the complementary experience of the 2 supervisors will ensure that the student receives expert input in all of the techniques utilized. Furthermore, Professor Bicknell has experienced members of his group that will provide “on spot help” as and when required as will members of Dr Martin’s proteomic group. Both supervisors will have regular contact with the student during the work at the level of day to day supervision and formal meetings will be held every week to monitor progress. Feedback from the student about the quality of training will be taken at these meetings and any changes required will be carried out.

The student role.

The Student will be trained to isolate the endothelial cells from tissues in order that they can prepare their own samples for the purification of cell surface proteins. The protocols to perform this work are performed routinely in the Bicknell group and training will be given by subject specific experts. Similarly the student will receive a thorough training in the protein biochemistry elements of the work to be able to isolate the surface proteins from the endothelial cells, to generate and isotopically label tryptic peptides. These peptides will be separated by reverse phase HPLC and sprayed on-line directly into an ESI source on a ToF/ToF mass spectrometer. The mass spectrometer will perform an automated MS MS/MS procedure so that the peptides are fragmented and can be identified. The student will be shown how to do this and how to search the data to identify and quantify the proteins and to assess the quality of the data. Therefore, the student will be responsible for all aspects of the work and in doing so will be fully trained in a range of fundamental techniques that will be widely applicable in future work.
### Project Outline

Problems during DNA replication are thought to be a major cause of the genomic instability that leads to cancer. Moreover, the process of genome replication is of vital importance to cancer progression; it is exploited by the frequent treatment of cancer with radiotherapy and drugs that target DNA replication. These drugs can be very effective but do not only target cancer cells and therefore elicit significant side effects. It is therefore crucial that we fully understand this fundamental process to understand the development of cancer and create novel therapies that target aspects of DNA replication that are deregulated in cancer cells.

Recently, inhibition of the earliest stages of DNA replication: licensing of replication origins and activation of replicative helicase, has been shown to preferentially kill cancer cells over normal ones. Replicative helicase is a complex of proteins, which unwinds double stranded DNA to expose single strands that act as templates during the replication process. We still know very little about the mechanism of helicase activation despite it being of a highest interest as obviously inhibitors of various parts of this mechanism should provide promising anti-cancer therapies.

My preliminary data suggest a particular order of events required for replicative helicase activation. The aim of this project is to determine the steps of this transformation and to test a restricted number of candidate proteins likely to be essential to drive this process.

To conduct this research, the Student will use a cell-free system that recapitulates a whole round of DNA replication *in vitro* and thus is invaluable for biochemical studies of eukaryotic DNA replication and DNA repair processes.

### Further reading:

<table>
<thead>
<tr>
<th>How are you planning to ensure adequate supervision?</th>
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<tbody>
<tr>
<td>I will supervise the Student myself on a day to day basis and teach the student all the techniques required. I will make sure that the Student understands the project in depth, that her / his lab book is kept up to date and that we discuss the progress of the project on the regular basis. Finally, I plan to ask the student to prepare a number of presentations about different aspects of the project to ensure that he / she gathers all required literature background knowledge over the duration of the project rather than leaving it till the end.</td>
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<tr>
<th>The student role.</th>
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<td>I expect the Student to become proficient in all the techniques he / she will need to use and to be able to carry on the experiments by him / herself after the initial training. All of the work carried out by the Student for the purpose of this project is laboratory based (wet science). All of the research conducted by the Student will be novel and hopefully will result in creating data that will be used for future grant applications and publications. The Student will be a co-author of any publication resulting from this project.</td>
</tr>
</tbody>
</table>
Lead Supervisor: Dr Eva Petermann

Co Supervisor: Dr Rebecca M. Jones

Project Title: Role of BRCA1 in the cellular response to Gemcitabine

Department: School of Cancer Sciences

Contact Email: e.petermann@bham.ac.uk
Telephone: 41 49165

Is the project cancer related? Yes

<table>
<thead>
<tr>
<th>Discipline</th>
<th>Cancer Sciences</th>
<th>Immunology</th>
<th>Pathology</th>
<th>Anatomy</th>
<th>Metabolic Medicine</th>
<th>Endocrinology</th>
<th>Metabolic Medicine</th>
<th>Liver &amp; GI Medicine</th>
<th>Haematology</th>
<th>Evolutionary biology in Clinical medicine</th>
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Project Outline

Purpose
Familial breast and ovarian cancers caused by mutations in the BRCA1 gene have defects in DNA damage repair, which affects the tumour response to DNA-damaging chemotherapy. This project investigates the role of BRCA1 in the response to the antimetabolite Gemcitabine, which is still poorly understood.

Background
Familial forms of breast cancer can be mutated in the BRCA1 or BRCA2 breast cancer susceptibility genes. Since BRCA1 and BRCA2 are components of the important homologous recombination DNA repair pathway, cancers bearing such mutations are less able to repair damaged DNA. This is important for therapy because many anti-cancer treatments work by damaging DNA. Patients with and without mutations in BRCA1 or BRCA2 do therefore respond differently to some treatments. Chemotherapy of breast cancer uses agents called antimetabolites such as Gemcitabine. Gemcitabine inhibits the progression of DNA replication forks (replication fork stalling), which induces DNA damage and kills cells. How cancers with different mutations respond to DNA damage induced by Gemcitabine is not well understood.

Our lab is interested in how Gemcitabine treatments induce DNA damage and how this damage is either repaired or causes cell death. Our recent work has shown that transient treatments with Gemcitabine, such as used in the clinic, induce irreversible replication fork stalling and more toxic types of DNA damage than the continuous treatments that have been used in previous in vitro studies. Surprisingly, cells with mutations in BRCA2 are more resistant to transient Gemcitabine treatments, and we are currently investigating the mechanism for this. BRCA1 performs some similar but also many different functions to BRCA2, and our new findings emphasise the need for investigating the role of BRCA1 in the cellular response to Gemcitabine as well.

Project objectives
1. Does BRCA1 status affect cellular survival of Gemcitabine treatments?
2. Does BRCA1 status affect replication fork stalling and DNA damage formation after Gemcitabine treatment?
Scientific approach
You will use BRCA1-mutated and wtBRCA1-complemented human breast cancer cell lines HCC-1937 and HCC-1937+BRCA1, and BRCA1 siRNA depletion in MCF-7 cells (BRCA1-proficient breast cancer cell line).
You will treat the cells with Gemcitabine and use clonogenic survival assays to investigate the effect of BRCA1 on cellular survival of this drug. The effect of BRCA1 status on replication fork stalling will be measured using the DNA fibre method, using thymidine analogues (CldU, IdU) to label nascent DNA in cells followed by immunofluorescence microscopy. Cells will be labelled with CldU for 20 minutes followed by incubation with replication inhibitors. Inhibitors will be washed out and restart allowed in presence of IdU. Chromosomal DNA will be spread on microscope slides and labelled DNA detected with specific antibodies. This will allow quantification of differences in frequency and/or speed of replication fork restart after Gemcitabine treatment.
To assess how BRCA1 status affects DNA damage, you will treat cells with Gemcitabine and perform immunofluorescence microscopy for DNA damage foci (γH2AX and 53BP1) and analyse DNA break formation by pulse-field gel electrophoresis.

Key references

How are you planning to ensure adequate supervision?
The student will be supervised both by the PI and Dr Rebecca Jones, a postdoctoral researcher in the lab with expertise in all major techniques used in this project. The PI will introduce the student into the project, hold weekly meetings with the student and operate an open-door policy for the rest of the week. Dr Jones will supervise the student on a day-to-day basis. The student will be able to obtain further expert support from the lab technician. The lab holds weekly meetings were results are presented to the group and feedback obtained. In addition, the PI meets with each lab member individually on a weekly basis. The student will be encouraged to work on drafting the final thesis over the course of the project and regularly submit drafts to the PI to obtain feedback.
Our group is part of a larger cluster of research groups with extensive expertise in all areas of DNA damage and genome stability, which hold weekly meetings and will provide further opportunities of support for the student. In the course of this project we will particularly interact with the lab of Dr Jo Morris.
The PI has access to two mentors (Martin Rowe and Tanja Stankovic) to help with any issues regarding supervision.
**The student role.**

The student will be expected to familiarise themselves with the background and purpose of the project and the key literature in the field before and during the course of the project. With the help of the supervisor and group members, she/he will learn central laboratory methods in mammalian cell culture and molecular biology of the DNA damage response. With support from the lab, the student will then apply the learnt methods to new experiments, and analyse and interpret the data obtained. She/he will keep a constant record of experiments conducted and results obtained, and regularly present the work to the group in lab meetings. The student is expected to develop increasing autonomy during the course of the project, take ownership of the project as much as possible, and be able to write a small thesis at the end. The student should spend all of the allocated time working on the project. The student will be expected to work responsibly as part of a team, honour the rules of the lab, and immediately report any problems encountered to the supervisor.
**Lead Supervisor:** Dr Neena Kalia

**Co Supervisor** Professor Gerard Nash

**Project Title:** Does coating stem cells with platelet microparticles enhance their adhesion to blood vessels

**Department:** Clinical and Experimental Medicine, Cardiovascular Sciences

**Contact Email:** n.kalia@bham.ac.uk

**Telephone:** ext : 58818

**Is the project cancer related?** Yes or No

**Discipline:**
- Cancer Sciences
- Immunology
- Pathology
- Anatomy
- Metabolic Medicine
- Endocrinology
- Haematology
- Liver & GI Medicine
- Infection
- Evolutionary biology in Clinical medicine

**Project Outline**

**Stem Cell Therapy for Hepato-Intestinal Disorders**

The bone marrow contains haematopoietic (HSC) and mesenchymal (MSC) stem cells (SCs). Both stem cell types can aid in the repair of a number of injured organs, including the liver and gut, gaining them clinical interest in the field of regenerative medicine. Although HSCs and MSCs can migrate to injured liver and gut and aid in tissue repair, clinical success remains poor. This has been partially explained by low numbers of SCs actually adhering to blood vessels within these organs when injected systemically, thus limiting their clinical utility. Developing strategies that enhance the adhesion of SCs as they circulate through injury sites could potentially confer greater therapeutic benefit. The Kalia group has been working on identifying ways of enhancing HSC/MSC recruitment within injured gut, liver and kidney. It is anticipated that increasing their recruitment will lead to more efficient and rapid tissue repair.

**Using Platelet Microparticles to Enhance Stem Cell Adhesion**

Platelet microparticles (PMPs) are small membrane vesicles (0.1-1μm) that are shed from the surface of platelets when they are activated ie. during thrombus formation. They are found circulating in abundance in patients with inflammatory disorders. Although initially considered cell debris, a central role in haemostasis has been identified for PMPs. More recently, studies demonstrated that they could also attach to inflammatory cells, such as neutrophils, and encourage their adhesion. We have demonstrated that PMPs can also ‘coat’ mouse HSCs and enhance their adhesion in vitro to endothelial cells (ECs) and in vivo to colitis injured mouse colon. We would now like to determine whether this strategy also works for enhancing murine MSC adhesion and also human SC adhesion as the latter would have greater clinical relevance. The limited studies published to date have attempted to manipulate SCs genetically in order to enhance their recruitment. However, since this study will aim to utilise a more biological approach to enhance SC adhesion, it may have greater translational applicability.
Aims of the Project

This project will therefore involve:

(i) **Generating murine and human PMPs.** Different agonists will be used to activate peripheral blood platelets to see which stimulus generates the most PMPs.

(ii) **Coating murine MSCs or commercial human SCs with PMPs.** Flow cytometry and confocal microscopy will be used to assess whether SCs and PMPs are actually physically interacting with (sticking to) each other. SCs will be incubated with various concentrations of PMPs to see if we can increase the binding between the two cell types.

(iii) **Determining SC adhesion to liver and colon ECs *in vitro.*** ECs will be cultured on glass capillaries or coverslips and the adhesion of PMP coated and non-coated SCs to them will be monitored under flow (mimics blood flow) or static conditions. The effects of increasing the PMP/SC interactions on SC adhesion to endothelium will also be determined.

(iv) **Determining the mechanisms by which any enhanced adhesion is mediated.** PMPs may enhance SC adhesion by increasing the ability of their surface adhesion molecules to bind to endothelial counterligands (ICAM-1, VCAM-1, HA, MadCAM-1) with greater affinity or they may induce surface clustering of adhesion molecules so that they bind more strongly. This will be determined using static adhesion assays and fluorescent confocal microscopy.

References:

How are you planning to ensure adequate supervision?

Although there will be weekly lab meetings with the Kalia and Nash groups, both supervisors will be available on a daily basis. Postdocs and research students from these groups will also ensure day-to-day supervision and guidance is provided.

The student role.

The student will work closely with a small group of researchers working on stem cell homing to injured organs. They will learn established methodologies including static and flow based adhesion assays, tissue culture, flow cytometry and fluorescent confocal microscopy. Although they will be closely supervised on arrival, they will be expected to work more independently as the project develops. They will present their data and contribute at weekly lab meetings and, if possible, present at a national conference. It is expected that the student will generate sufficient data for one paper. There are a number of local weekly seminars (Cardiovascular, VITA, stem cell seminars etc) that students will be encouraged to attend as part of their training in scientific research.
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<tr>
<th><strong>Lead Supervisor:</strong></th>
<th>Mr Chris Coulson</th>
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<td><strong>Co Supervisor:</strong></td>
<td>Professor Joanne Wilton</td>
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<tr>
<td><strong>Project Title:</strong></td>
<td>Cochlear implantation: Temporal bone anatomy imaging and surgery</td>
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<tr>
<td><strong>Department:</strong></td>
<td>ENT, QEH and Department of Anatomy, Medical School</td>
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<tr>
<td><strong>Contact Email:</strong></td>
<td><a href="mailto:j.c.wilton@bham.ac.uk">j.c.wilton@bham.ac.uk</a></td>
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<tr>
<td><strong>Telephone:</strong></td>
<td>0121 414 6838</td>
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<td><strong>Is the project cancer related?</strong></td>
<td>No</td>
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**Discipline:**
- Cancer Sciences
- Pathology
- Metabolic Medicine
- Haematology
- Infection
- Immunology
- Anatomy
- Endocrinology
- Liver & GI Medicine
- Evolutionary biology in Clinical medicine

### Project Outline

Patients with profound bilateral sensorineural hearing loss (usually cochleogenic) are imaged prior to cochlear implantation. Typically, both CT and MRI scans are performed. This project aims to determine the pick up rate of abnormalities on these imaging modalities and whether we can rationalize our imaging strategy by producing a protocol based on the patient’s individual symptoms. The project will involve an ENT surgeon (Mr Coulson) and a radiologist (Dr Colley) who will teach the student how to interpret CT and MRIs and how to look for specific findings on all of the scans from our cochlear implant patients (currently 1300). These findings will be linked this with operative findings and, potentially, audiological outcomes.

This project would give the student an excellent insight into temporal bone anatomy through dissection of lateral face and temporal bone (in anatomy) and this will be supplemented with attendance in theatre to watch cochlear implantation.

**How are you planning to ensure adequate supervision?**

The students will be working closely within and supervised by ENT clinical and research staff and anatomy prosectorium staff and technicians throughout. Additionally we will arrange once weekly meetings with supervisors.

**The student role.**

The students will be taught all appropriate methodologies and skills, but will be expected to work independently in collating data and writing reports. The student must be of a temperament that enjoys meticulous working methods (and accept the time required to do such work thoroughly) both in dissection and on computer screens. The student is likely to have an interest in a possible career in surgery or radiology and an interest especially in head and neck anatomy.
**Lead Supervisor:** Mr Adrian Gardner

**Co Supervisor**
Professor Joanne Wilton

**Project Title:** Safe instrumentation patterns for growing rod surgery in correction of spinal curvature

**Department:** Orthopaedics, ROH and BCH and Department of Anatomy, Medical School

**Contact Email:** j.c.wilton@bham.ac.uk

**Telephone:** 0121 414 6838

**Is the project cancer related?** No

**Discipline:**
- Cancer Sciences
- Pathology
- Metabolic Medicine
- Haematology
- Infection
- Immunology
- Anatomy
- Endocrinology
- Liver & GI Medicine
- Evolutionary biology in Clinical medicine

**Project Outline**

Congenital malformations of the spine are often corrected surgically by placement of spine rods. In children however growth may be restricted and novel methodologies are being developed to allow growth but also correct a scoliosis. One growing spine rod has been developed where the rod is fused only at the top and bottom of a scoliosis curve and then serially distracted to stimulate spinal growth with curve control. However several case reports describe the have pulled off and the fixing screws have transacted the cord resulting in paralysis.

The project aims to test different types of instrumentation (screws, hooks, wires) used in in the thoracic spine and modes and strengths to failure so that the safest instrumentation pattern can be developed. It would require dissection of spine, instrumentation, potting and then mechanical testing with CT imaging pre and post testing.

This project would give the student an excellent insight into spinal anatomy through dissection and viewing of CT images - this will be supplemented with attendance in theatre to watch corrective surgery.

**How are you planning to ensure adequate supervision?**

The students will be working closely with and supervised by Mr Gardner and orthopaedic research staff and Professor Wilton and prosecting technicians throughout. Additionally we will arrange once weekly meetings with supervisors.

**The student role.**

The students will be taught all appropriate methodologies and skills, but will be expected to work independently in collating data and writing reports. The student must be of a temperament that enjoys meticulous working methods (and accept the time required to do such work thoroughly). The student is likely to have an interest in a possible career in surgery or radiology and an interest especially in spinal anatomy and neuroanatomy.
Project Title: Femoral splint placement

Department: T&O, QEH and Department of Anatomy, Medical School

Is the project cancer related? No

Discipline: Cancer Sciences [ ] Pathology [ ] Immunology [ ]
Cancer Sciences [ ] Pathology [ ] Immunology [ ]
Cancer Sciences [ ] Pathology [ ] Immunology [ ]
Cancer Sciences [ ] Pathology [ ] Immunology [ ]

Project Outline

Appropriate placement of splints prior to hospitalisation of trauma patients is critical to stabilisation prior to transport and helps reduce the amount of hospital treatment and reduce recovery times and outcomes. Femoral splints are commonly used following trauma by paramedics as well as in hospital to stabilise fractures.

This project aims to investigate the amount of movement in pre-hospital and hospital placed femoral splints especially in counter-traction stresses where there is some evidence of medial displacement of splints and potential damage to soft tissues and fracture repair. Part of the project will look at counter-traction pelvic and femoral stresses in cadaveric specimens, and part will review hospital imaging library of splints and fractures in patients with pelvic and lower limb trauma.

It is hoped that this project will lead to more accurate information on splint placement for paramedics well as trauma departments to benefit outcome for these patients.

This project would give the student an excellent insight into pelvic and lower limb Anatomy through dissection as well as an understanding of load bearing in these structures. There will be investigative work with cadaveric specimens and review of radiological images on arrival and post-treatment of patients with pelvic and femoral fracture. Students will also have the opportunity to observe surgery of such complex fractures in theatre.

How are you planning to ensure adequate supervision?

The students will be working closely within and supervised MR Cooper and by appropriate T&O clinical and research staff and by Professor Wilton for cadaveric studies with the help of anatomy prosectorium staff and technicians. Additionally we will arrange once weekly meetings with supervisors.
The student role.

The students will be taught all appropriate methodologies and skills and will be encouraged to consider development of new assessment protocols, there will also be independent work in collating data and writing reports. The student must be of a temperament that enjoys meticulous working methods (and accept the time required to do such work thoroughly) both in dissection and on computer screens in reviewing radiological images. The student is likely to have an interest in a possible career in surgery or radiology and an interest especially in musculoskeletal system/orthopaedics.
<table>
<thead>
<tr>
<th><strong>Lead Supervisor:</strong></th>
<th>Mr Matthew Trotter</th>
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<tr>
<td><strong>Co Supervisor</strong></td>
<td>Professor Joanne Wilton</td>
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<tr>
<td><strong>Project Title:</strong></td>
<td>Variations in venous drainage from skull base to mediastinum</td>
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<tr>
<td><strong>Department:</strong></td>
<td>ENT Heartlands Hospital and Department of Anatomy, Medical School</td>
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<td><strong>Contact Email:</strong></td>
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**Is the project cancer related?**  No

**Discipline:**

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<td>Liver &amp; GI Medicine</td>
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<tr>
<td>Infection</td>
<td>Evolutionary biology in Clinical medicine</td>
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**Project Outline**

This project aims to study CT images as well as dissection studies to investigate the normal variation in the vascular course and distribution in the neck with particular emphasis on venous drainage from skull base to mediastinum and number with dominant venous drainage on one side. This project may expand to again use CT images to investigate constitutional variance within the sigmoid/jugular bulb venous drainage.

A meticulous neck and skull base dissection will form part of this study to demonstrate the superficial and deeper structure within the neck and their relations to the cranial base.

This project would give the student an excellent insight into neck anatomy through CT imaging and dissection and this may be supplemented with attendance in theatre to observe surgery.

**How are you planning to ensure adequate supervision?**

The students will be working closely within and supervised by Mr Trotter and radiology staff at Heartlands hospital and with Professor Wilton and prossecting technicians for the dissection component. Additionally we will arrange once weekly meetings with supervisors.

**The student role.**

The students will be taught all appropriate methodologies and skills, but will be expected to work independently in collating data and writing reports. The student must be of a temperament that enjoys meticulous working methods (and accept the time required to do such work thoroughly). The student is likely to have an interest in a possible career in surgery or radiology and an interest especially in head and neck anatomy.
Blood flow through human vessels appears to be in a longitudinal vector (laminar) as well as in a transverse vector-in effect this makes the blood flow in a spiral pattern. This type of movement conserves energy and stabilises the flight of projectiles—a bullet for instance. Likewise the flow of blood is propagated forward and the forces on the vessel wall are stabilised. Spiral laminar flow is hypothesised as protective of the endothelium which is critically dependent upon a certain range of wall shear stresses to keep it healthy. In areas where disturbed flow occurs (e.g. carotid bifurcation, iliac bifurcation) increased vascular disease occurs.

Spiral flow is though to be generated centrally by the heart and the structure of the aorta. We have hypothesised that peripheral vessel wall also acts to generate spiral flow through obliquely arranged muscle fibres. From preliminary cadaveric studies the arrangement of muscle fibres in arteries and some veins appears to be oblique rather than circular and this would be supportive of the hypothesis. Further studies are required to assess this, particularly in other vessels and in fresh tissue using simple histological assessments.

The study is pending Biobank approval but should allow serial sections and angle sections to be processed and assessed from fresh tissue and potentially donor cadaveric tissue following this.

The justification of the work is that vessel disease is common in arteries and veins. The relevance of this is in early identification of abnormal flow predicting disease and design of intravascular devices (grafts/stents/lines etc) where generation of spiral flow may reduce the high rate of failure.

How are you planning to ensure adequate supervision?

The students will be working closely within and supervised by Mr Inston and clinical research staff and also anatomy prosectorium staff and technicians throughout. Additionally we will arrange once weekly meetings with supervisors.
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<th><strong>The student role.</strong></th>
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Background: Misregulation of gene expression is a major mechanism of multifactorial diseases. The identification of common genomic sequence variants that contribute to human multifactorial and inherited disorders remains a significant challenge. Such can occur due to mutations in protein coding genes as well as cis-regulatory modules and other non-coding effectors of gene expression. However, little attention has been given to non-coding mutations involved in disease and disease susceptibility. Genome wide association studies indicate that many disease susceptibility loci are in candidate cis-regulatory elements (such as enhancers). The recent discoveries of the human ENCODE project emphasized the importance of the millions of predicted enhancers and other cis-regulatory elements present in the human genome. However, functional relevance and general understanding of the mechanisms of function of the these enhancers around genes remains lacking. The functional testing of these candidate enhancers can only be achieved in suitable transgenic animal models in which a reporter gene attached to the candidate elements is introduced and the regulatory potential revealed by analysis of reporter gene expression. It was previously demonstrated that many functionally important enhancers are conserved between species of large evolutionary distance such as human and zebrafish, which have diverged for over 450 million years. Yet, sequence conservation indicates that developmental regulatory processes are retained between fish and human and makes zebrafish a suitable animal model for testing human enhancer function. Zebrafish is a cost effective and simple vertebrate model in which transgenesis can be carried out at relative ease. The transparent, externally developing embryo provides convenient means to monitor fluorescent reporter gene expression in vivo and the large number of embryos that can be rapidly injected can be imaged by automated high throughput imaging system developed by the host laboratory (Gehrig et al., 2009)

Project aim: to generate gain and loss of function evidence for predicted human enhancers associated with T2 diabetes.

Methods: 1.) generation of reporter gene expression vector including human enhancer candidates 2.) Microinjection of zebrafish embryos with reporter expression vectors 3.) monitoring of reporter activity by fluorescence microscopy 4) comparison of activity of wild type enhancers with SNPs associated with T2D.

The constructs will be injected with a site specific integrase system (PhiC31) to avoid
position effects and allow direct comparison and quantitative analysis of reporter activity in transgenic zebrafish embryos. The expected outcome of this work is the functional validation of a set of cios regulatory elements associated with disease and a functional genomic proof of principle for a disease causing mechanism.

**References:**


### How are you planning to ensure adequate supervision?

The proposed project is funded by Framework 7 programme of the European Commission, and allows the student to interact with colleagues from partner laboratories all across Europe. The project is integral to the central research focus of the laboratory and the student will have ample opportunity to interact with several colleagues working on related projects. Daily supervision and training in techniques will be provided by the experienced post doc Dr Y. Hadzhiev and I. Miguel. Weekly discussion about the project between both supervisors and student is implemented to plan consecutive experiments to monitor progress and evaluate outcomes. The main supervisor will guide in writing the thesis.

### The student role.

The student will train in the theory, experimental design and experimental techniques required for the successful outcome of the project, to carry out experiments designed together with supervisors and to give oral progress reports in labmeetings aided by ppt presentations. All aspects of the project can be trained within the time frame and the student will be able to carry out each segment of the work in a semi-independent fashion. The student will then write a thesis and generate figures that may be suitable for use in a planned scientific peer reviewed publication with coauthorship to the student. The project will thus provide a headstart for further training/potential career in the academic environment.
Primary Supervisor: Dr Ferenc Mueller

Project Title: Function of TBP associated factor 8 (TAF8) in mediating PPAR-dependent gene expression and lipogenesis in the zebrafish embryo model

Department: School of Clinical and Experimental Medicine, College of Medical and Dental Sciences

Contact: f.mueller@BHAM.AC.UK, 42895

Discipline: Cancer Sciences, Pathology, Metabolic Medicine, Haematology, Infection, Immunology, Anatomy, Endocrinology, Liver & GI Medicine, Evolutionary basis of clinical medicine

Project Outline

The nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR\(^\gamma\)) regulates the process of intracellular neutral lipid accumulation (lipogenesis) in formation of white fat tissue as well as in several organs including the liver and muscle. PPAR\(^\gamma\) plays multiple roles in lipid physiology and when misregulated lead to various diseases associated with fat storage. Understanding the molecular mechanisms of PPAR\(^\gamma\) function may lead to identification of drug targets and development of novel drugs. Recent data in various components of the basal transcription initiation machinery.

Specialisation of cell types (differentiation) is in part accomplished by a specialization of the basal transcription machinery, which is required for the recruitment of Polymerase II during transcription initiation of regulated genes. TBP associated factors (TAFs) are hypothesized to be specific mediators of regulatory input from transcriptional activators (reviewed in ¹). However, little is known about the specificity of TAFs in interacting with transcriptional activators.

The zebrafish has recently emerged as a versatile vertebrate animal model for human disease by virtue of its fast, transparent and extra-utero development, which is amenable to manipulation and visualization of phenotypes upon interference with genetic and cellular processes. ². Using the zebrafish model, we propose to address the predicted specific roles of TAF8 in mediating PPAR\(^\gamma\) signaling ³. We have shown that PPAR\(^\gamma\) binds TAF8, and that TAF8 is required for lipogenesis in zebrafish embryos. In this project, we will modulate PPAR signaling by agonists and antagonists and detect lipogenesis by in situ staining techniques in liver and other organs of the transparent zebrafish embryo during development. We will carry out genetic interaction analysis using TAF8 and TAF6 mutants and analyse candidate target gene expression by whole mount in situ hybridization. It is expected that the experiments will provide conclusive evidence to the specific role of TAF8 in mediating PPAR\(^\gamma\) signals in lipogenesis of embryos. The project will provide training in work with an animal model, embryo anatomy and development, embryo phenotyping and gene expression analysis. The project requires Home Office training modules 1-3.

¹ Muller, F., Zaucker, A. & Tora, L. Developmental regulation of transcription initiation: more than just changing the actors. *Curr Opin Genet Dev* **20**, 533-
How are you planning to ensure adequate supervision?

Daily supervision and training in techniques will be provided by the experienced post doc Dr Emma Kenyon. Weekly discussion about the project between both supervisors and student is implemented to plan consecutive experiments to monitor progress, and evaluate outcomes. The main supervisor will guide in writing the thesis.

The student role.

To train in the experimental techniques required for the project, to carry out experiments designed together with supervisors and to give oral progress reports in lab meetings aided by ppt presentations. To write a thesis and generate figures that may be suitable for use in a planned publication which may benefit from the outlined project.
**Lead Supervisor:** Dr Heather Long

**Co Supervisor**

Dr Graham Taylor

**Project Title:** CD4+ T cells and the control of EBV-positive post-transplant lymphoproliferative disease

**Department:** School of Cancer Sciences

**Contact Email:**

h.m.long@bham.ac.uk

**Telephone:**

0121 414 2808

**Is the project cancer related?** Yes or No

**Discipline:**

- Cancer Sciences
- Pathology
- Metabolic Medicine
- Haematology
- Infection
- Immunology
- Anatomy
- Endocrinology
- Liver & GI Medicine
- Evolutionary biology in Clinical medicine

**Project Outline**

It is now clear that in many settings CD4+ T cells can directly recognise and kill MHC class II-positive target cells expressing their cognate antigen. For malignancies such as lymphoma occurring within constitutively MHC II-expressing cells, this opens up exciting possibilities for therapeutic exploitation of CD4+ T cells. In this context, Epstein-Barr virus (EBV)-associated post-transplant lymphoproliferative disease (PTLD) can be cured by adoptive transfer of T cells generated by *in vitro* stimulation with EBV-transformed lymphoblastoid cell lines (LCLs; virus-infected cells that resemble the tumour cells that outgrow in PTLD). In a recent study, PTLD patients who receive infusions of 3rd party LCL-stimulated T cell preparations containing higher percentages of CD4+ T cells achieved better long-term clinical responses. However, the CD4+ component of the blood is phenotypically and functionally heterogeneous, and the identity and functional characteristics of the CD4+ T cells present remains to be determined.

This project will use multi-colour flow cytometric analysis and our novel MHC class II tetramers to assess the specificity and characteristics of the CD4+ T cells present in the blood of healthy donors and expanded in the LCL-stimulated preparations used therapeutically. A better understanding of these crucial immune effectors and their relevant immune targets may eventually lead to swifter preparation of therapeutic T cell lines against more clinically relevant antigenic targets, and hence improve the future long-term survival of patients with B cell malignancies.

**Key References:**


<table>
<thead>
<tr>
<th>How are you planning to ensure adequate supervision?</th>
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<tbody>
<tr>
<td>This project will be supervised by Dr Heather Long who will teach the student the required techniques and be available to meet on a daily basis. Dr Long has expertise in EBV T cell immunology and multicolour flow cytometry and has a long-term interest in this research area. Additionally, the student will also be supported by Dr Graham Taylor who has expertise and a direct interest in this work, and will be available to meet with the student.</td>
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<table>
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<tr>
<th>The student role.</th>
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<tr>
<td>The student will work alongside Dr Heather Long, Dr Graham Taylor and other members of the EBV immunology team. The project will provide the student both with training in basic laboratory techniques, and with practical experience of several fundamental immunology techniques. Specific laboratory techniques that will be involved in the project include lymphocyte isolation, cell culture, ELISAs and multi-colour flow cytometry. During the project he/she will become proficient in the design and execution of appropriately controlled experiments. Additionally, the student will have the opportunity to further their knowledge of viral and tumour immunology by attending group meetings, journal presentations and seminars given by members of the institute and visiting international researchers.</td>
</tr>
</tbody>
</table>
**Lead Supervisor:**  Dr Heather Long

**Co Supervisor**  
Dr Graham Taylor

**Project Title:**  T cell targeting of lymphoma

**Department:**  School of Cancer Sciences

**Contact Email:**  
[LINK]

**Telephone:**  
0121 414 2808

**Is the project cancer related?**  Yes

**Discipline:**  
- Cancer Sciences
- Immunology
- Pathology
- Anatomy
- Metabolic Medicine
- Endocrinology
- Haematology
- Liver & GI Medicine
- Infection
- Evolutionary biology in Clinical medicine

### Project Outline

The rationale for T cell therapy for cancer is that tumour cells express a different repertoire of proteins, compared to healthy tissue, which may be targeted by the immune response. The clinical potential of T cell-based therapy has been best illustrated in the successful treatment of virus-associated malignancies, such as Epstein-Barr virus (EBV)-associated post-transplant lymphomas (PTLD). This fatal lymphoproliferative disease may be reversed by adoptive-transfer of virus-specific T cell preparations. However, many malignancies are not associated with viral infection, and T cell therapy relies on immune targeting of cellular antigens. We have recently shown that EBV transformation leads to up-regulation of not only viral antigens, but also cellular antigens that can be recognised by the CD4+ immune system. Importantly, these cellular targets are also expressed in at least some other non-virus-associated lymphomas, and may provide novel therapeutic targets for lymphoma. We are now interested to investigate the CD8+ T cell subset for the similar presence of effector T cells that recognise cellular antigens up-regulated by EBV transformation, and determine whether these can also recognise other non-virus transformed lymphoma cells.

### Key References:


### How are you planning to ensure adequate supervision?

This project will be supervised by Dr Heather Long who will teach the student the required techniques and be available to meet on a daily basis. Dr Long has expertise in T cell isolation, immunological assays and multicolour flow cytometry and has a long-term interest in this research area. Additionally, the student will also be supported by Dr Graham Taylor who has expertise and a direct interest in this work, and will be available to meet with the student.
**The student role.**

The student will work alongside Dr Heather Long, Dr Graham Taylor and other members of the tumour immunology team. The project will provide the student both with training in basic laboratory techniques, and with practical experience of several fundamental immunology techniques. Specific laboratory techniques that will be involved in the project include lymphocyte isolation, cell culture, ELISAs and multi-colour flow cytometry. During the project he/she will become proficient in the design and execution of appropriately controlled experiments. Additionally, the student will have the opportunity to further their knowledge of viral and tumour immunology by attending group meetings, journal presentations and seminars given by members of the institute and visiting international researchers.
### Project Outline

*Streptococcus pneumoniae* (the pneumococcus) causes important diseases of man including pneumonia and meningitis. The pneumococcus produces virulence factors including a toxin and several surface–associated enzymes that allow the organism to cause disease. One of these enzymes is a neuraminidase that cleaves sialic acid from host glycoproteins. It has been shown that this neuraminidase plays a key role in the attachment to brain endothelial cells and mediates transcytosis of brain endothelial cell layers (Uchiyama et al., 2009). The sialic acid released from host cells may act as a signalling molecule the alter gene expression in the pneumococcus to allow this interaction to occur. For example, sialic acid is known to be involved in signalling pathway causing the pneumococcus to form biofilms (Trappetti et al., 2009). The aim of the project is to investigate the mechanism by which neuraminidase promotes interaction of the pneumococcus with human brain endothelial cells. The role of the enzymatic activity of the toxin in attachment and invasion of cells will be investigated by use of neuraminidase inhibitors. The role of sialic acid will be determined by evaluation of the effect of exogenous sialic acid on attachment, and the effect on gene/protein expression by the bacterium. This will determine whether other adhesins may also be up-regulated by the released sialic acid. These studies will allow us to develop new strategies to prevent pneumococcal infections.


### How are you planning to ensure adequate supervision?

- The project is designed on a ‘modular’ basis with defined outcomes so that results will be generated early in the project and more ‘high risk – high return’ elements are done at the end
- Project progress is monitored by weekly scheduled meetings with primary supervisor
- Secondary supervisor is available for day-to-day lab supervision
- Input from other members of research team (2 further post-docs, 3 PhD students and 1 technician)

### The student role.

The student will learn to grow human cell lines for the blood-brain barrier model and will use this model to measure bacterial adhesion and transcytosis (ability to cross the endothelial cell layer. The student will run enzyme assays to determine the optimal concentrations of neuraminidase inhibitors to use in the cell assays to block bacterial neuraminidase activity. The student will determine the effect of exogenous sialic acid on adhesion and transcytosis, and on expression of the bacterial neuraminidase using Real-Time PCR to measure gene expression and Western Blotting to monitor protein production. If time permits the student will determine the effect of sialic acid or presence of human endothelial cells on total gene expression in the pneumococcus using RNA sequencing approaches. If this is not possible existing datasets will be used to train the student in bioinformatics associated with this approach.
**Lead Supervisor:** Dr Sarah Leonard

**Co Supervisor** Dr Sally Roberts

**Project Title:** Role of the epigenetic regulators, DNA methyltransferases, in high-risk human papillomavirus pathogenesis

**Department:** Cancer Sciences

**Contact Email:**
- Telephone: 0121 4149234 (S. Leonard)
- 0121 4147459 (S. Roberts)

**Is the project cancer related?** Yes

<table>
<thead>
<tr>
<th>Discipline</th>
<th>Cancer Sciences</th>
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**Project Outline**

**Background**

High-risk human papillomaviruses (HPV) types HPV16 and HPV18 are associated with cancers of the cervix and of the oropharynx. Deregulation of expression of the two major HPV oncoproteins, E6 and E7 is known to contribute to the progression of HPV infected cells to malignancy. One of the mechanisms by which E6 and E7 deregulation occurs is by integration of the viral genome into the host DNA, which leads to the loss of expression of the E2 protein, a negative regulator of E6 and E7 expression. The E6 and E7 proteins work in a synergistic fashion to deregulate cellular pathways controlling cell proliferation and survival. These functions are necessary for virus productivity, but in persistent HPV infections they can render cells genetically unstable culminating in immortalization, and in rare instances malignancy. Whilst HPV-induced genetic abnormalities clearly play a role in disease progression there is evidence that virus-induced epigenetic modifications may also contribute to disease progression. One such epigenetic modification is DNA methylation, which occurs within CpG dinucleotides and contributes to the regulation of gene expression in normal cells. However, both hypomethylation-associated oncogene activation and hypermethylation-associated tumour suppressor gene silencing can lead to cancer initiation and progression. We have recently shown that the detection of methylated forms of a tumour suppressor gene in cervical samples parallels the natural history of cervical HPV infection.

One of the mechanisms by which HPV may contribute to the epigenetic reprogramming of cells is by interacting with the enzymes which control DNA methylation, the DNA methyltransferases (DNMT): DNMT1, DNMT3A and DNMT3B. Whereas DNMT1 has a preference for hemimethylated DNA and is involved in the maintenance of methylation, DNMT3A and DNMT3B function as de novo methyltransferases. Increased expression of one or more of the DNMT has been reported at a number of sites of cancer and has been shown to be an adverse prognostic factor. In cervical neoplasia, DNMT1 and DNMT3B expression is deregulated, and we have shown that these expression changes are recapitulated in cell-based models of HPV infection. It has previously been shown that HPV16 oncoproteins E6 and E7 increase the expression and activity of DNMT1 in a
transformed cell line, and we have preliminary evidence indicating the oncoproteins also regulate not only DNMT1 but also DNMT3B in primary cells infected with high risk HPV types.

**Hypothesis**

HPV oncoproteins contribute to epigenetic changes of the host cell by targeting the DNA methyltransferases.

**Objective and Aims**

The objective of this project is to determine if HPV oncoproteins target the *de novo* DNA methyltransferases and investigate the significance of the targeting of these epigenetic regulators to HPV replication.

*Objective 1:* To examine the interaction between HPV oncoproteins and the DNMTs

One mechanism by which HPV may modulate DNA methylation is by binding to the DNMTs. The interaction between the HPV oncoproteins and the *de novo* methyltransferases DNMT3A and DNMT3B will be investigated using coimmunoprecipitation experiments. Whether binding between HPV oncoproteins and DNMTs is related to viral DNA integration status will be investigated using cell-based models that show HPV genome integration upon extended cell culture.

*Objective 2:* To determine the effect of HPV oncoproteins on DNMT expression and activity

The effect of HPV oncoproteins on the expression and cellular location of the DNMTs will be investigated in primary cells containing high risk HPV genomes, and in primary cells expressing E6 and E7 alone, using western blotting and immunohistochemistry. An effect of oncoprotein expression upon enzymatic activity of the DNMTs will be determined. The relationship between DNMT binding to HPV oncoproteins and any effect on DNMT will be assessed using mutant forms of the HPV oncoproteins.

*Objective 3:* Silencing of DNMT expression in HPV genome containing cells

To address the significance of HPV induced deregulation of the DNMTs to virus replication, the expression of individual DNMTs will be silenced in cells containing episomal and integrated forms of the HPV genome using siRNAs. The effect of DNMT silencing on virus replication and host cell functions, including cell proliferation will be assessed using Southern blotting and cell cycle analyses.

**References**


**How are you planning to ensure adequate supervision?**

The student will meet with the supervisors on a weekly basis to discuss progress and to plan experiments. Both supervisors will be available on a daily basis for laboratory supervision and the student will also benefit from informal supervision from other experienced group members. The student will attend the weekly meetings of the HPV groups (Roberts, Woodman); within this informal setting they will have the opportunity to present their findings to the groups and receive constructive feedback on experimental data and on presentation skills. The student will also attend a meeting with all HPV groups within the School of Cancer Sciences (Parish, Roberts, Woodman), which takes place every two months.

**The student role.**

The student will be trained in all of the relevant techniques required to deliver the project aims. They will be expected to discuss their findings in the context of the scientific literature relating to their project. The student will have the opportunity to contribute to the direction and development of the project.
Lead Supervisor: Dr Ye Htun Oo

Co Supervisor Dr Nick Murphy
Dr Tony Whitehouse

Project Title: Investigating of role of ammonia affecting lymphocyte subset function in fulminant liver failure (FLF)

Department: Centre for Liver Research & NIHR BRU, 5th Floor, IBR. UHB NHS Foundation Trust

Contact Email: y.h.oo@bham.ac.uk
Nick.Murphy@uhb.nhs.uk; Tony.Whitehouse@uhb.nhs.uk

Is the project cancer related? No

Discipline:
- Cancer Sciences
- Immunology
- Pathology
- Anatomy
- Metabolic Medicine
- Endocrinology
- Haematology
- Liver & GI Medicine
- Infection
- Evolutionary biology in Clinical medicine

Project Outline

An emerging role for ammonia affecting lymphocyte functions in the pathogenesis of acute failure has been described (seronegative fulminant liver failure and paracetamol overdose fulminant liver failure [1, 2]. Fulminant hepatic failure patients has high level of ammonia, pro-inflammatory cytokines and chemokines in the blood that alter the lymphocyte subsets phenotype, function and promoting their recruitment which leads to hepatocyte necrosis. [3]. We have recently shown that the balance between regulatory T cells and effector Th1 and Th17 cells is crucial in determining outcome in autoimmune hepatitis [4]. Our previous data suggested that lymphocytes are recruited to fulminant liver failure [5]. Now we like to investigate further by using the peripheral blood from 10 POD and 10 Seronegative FLF patients and their explant tissue.

Project outline

1) Phenotypic characteristic of lymphocyte subsets in FLF patients

2) Analyse the serum/plasma inflammatory cytokines, chemokines and NH3 level in FLF patients

3) To analyse the function of CD8 effector and CD4+CD25highCD127low regulatory lymphocyte in FLF patients.

4) Glutamine synthetase activity is reduced in FLF patients.

5) Increased effector cells infiltration via hepatic sinusoids and hepatocyte cell death in FLF patients.

Aim of the project

To investigate the role of ammonia on lymphocyte subsets functions in FLF.

Benefits: This project will allow the student to learn a range of laboratory techniques and to generate important new data about the pathogenesis of seronegative hepatitis, a leading cause of liver failure, and liver transplantation in UK. The results may suggest novel therapies aimed at lymphocytes in the treatment
Successful student will have an opportunity to apply for FALK bursary by end of February.

Techniques to be used in the project: Human cell culture and isolation, cells isolation from human peripheral blood B cells subsets from liver clinics and explanted liver tissue; phenotyping with flow cytometry, immunocytochemistry, confocal microscopy, qPCR, functional assays (co-culture, blocking assays, proliferation and survival assays).

How are you planning to ensure adequate supervision?
We have chosen to combine the technical expertise of two supervisors to ensure the student is exposed to the maximal number of transferable research skills. Dr Ye Htun Oo is dedicated to 80% of his time in the laboratory, which is well equipped and set up for this type of study. Dr Murphy and Dr Whitehouse are internationally renowned for his work in FLF and has a proven track record in supervision of both undergraduate and postgraduate students. The Centre for Liver Research is part of the MRC Centre for Immune regulation and includes a large number of scientists working on liver immunology so the student will be working in a stimulating and supportive environment providing the student with exposure to research staff at all stages of their research careers. Appropriate training in research techniques and data analysis and presentation will be provided. We have weekly meetings with supervisors and interactions with other members of the research groups, who will be available day to day for advice and tuition.

The student role.
The student will be expected to perform experiments and conduct data analysis. They will present data to the supervisors and team and wider research groups and be expected to assimilate available published literature under guidance from the supervisors. They will be trained and expected to work using good laboratory practice under local standard operating procedures and be mindful of ethical use of human tissue specimens. They will be working in a large research teams and thus would be expected to interact with colleagues and use local facilities in a respectful manner. They could have an opportunity to sit in and observe in dedicated autoimmune hepatitis clinics to correlate laboratory bench work findings to pathogenesis and investigation of hepatic inflammation and autoimmunity. This would provide the student with an opportunity to link basic science to the patient.
**Project Outline**

**Background**

The immune system offers a powerful tool in the fight against cancer principally because of the remarkable specificity that it affords. Antibodies for cancer therapy are already in clinical use, but T cells offer even greater potential in this context because of their ability to recognise intracellular antigens. Indeed, clinical studies have already highlighted the potential of T-cell based therapies for some cancers.\(^1\)\(^2\)\(^3\) Virus-associated malignancies are important models in the development of such therapies since they express defined foreign antigens that can be targeted by the host immune response.

Head and Neck squamous cell carcinoma (HNSCC) is the sixth most common cancer. Recent years have seen a marginal decline in the incidence of most HNSCCs, but the incidence of those that develop at the oropharynx has increased significantly. Much of this increase has been due to the rise of carcinomas carrying the Human Papilloma virus (HPV) (Figure 1). Indeed it is predicted that, unless preventative measures are introduced, in 2020 HPV+ HNSCC will outnumber cervical cancer in the USA.\(^4\) Patients with early (stage I/II) HNSCC respond well to treatment, but patients with advanced (stage III/IV) disease respond less well, and tumours often recur.\(^5\) Interestingly, HPV+ oropharyngeal cancer has a more favourable prognosis\(^6,7\) which may, at least in part, be a result of immune responses directed at the viral proteins expressed in the tumour cells.

**Figure 1.** 50-80% of Oropharyngeal carcinoma cases carry Human Papilloma virus (HPV) in the malignant cells.

Within HNSCC, HPV expresses two oncogenic proteins E6 and E7, that are also expressed in HPV+ cervical cancer.\(^8\) The presence of viral proteins within malignant...
cells should provide a specific target for immune-mediated rejection. Studies in HPV+ cervical cancer have demonstrated that the E6 and E7 oncoproteins can be targeted by T cells and a number of peptide epitopes have been defined, some of which are presented through common HLA alleles including HLA A2. Several studies have explored the immunology of HNSCC (reviewed \textsuperscript{10}), but at the time the importance of HPV infection was not widely appreciated and so the HPV status of the tumour and the virus-specific immune response were not determined. Therefore the contribution of HPV-specific T cell immunity in HNSCC remains largely unknown. Our limited understanding to date is from two studies that reported increased frequencies of circulating T cells specific for the E7 protein in HPV+ compared to HPV- HNSCC patients and healthy donors\textsuperscript{11,12}. Furthermore a recent study detected HPV-specific T cells in the tumour tissue of 6/8 HPV+ HNSCC patients\textsuperscript{13}. Clearly, however there is a need to explore this response more thoroughly, not only to determine the role it plays in the development and clinical management of the tumour, but also the potential for boosting such responses to treat patients.

**Aim:** To characterise the HPV-specific T cell response in the blood and tumour tissue of HNSCC patients.

This project brings together two research groups within the medical school, combining the expertise of Dr Steve Lee (T cell responses to virus-associated malignancies and development of T cell therapies for cancer) and Prof Hisham Mehanna (Consultant Head-Neck and Thyroid Surgeon who runs a large Head and Neck cancer clinic at the QE). Through Prof Mehanna’s clinic we are ideally placed to access large numbers of fresh tumour tissue and blood samples from HNSCC patients. With these samples we will explore the following questions:

1. **What type of T cell response is present at the tumour site of HPV+ HNSCC patients?** Using flow cytometry and in vitro assays of T cell function we will characterise the HPV-specific CD8+ and CD4+ T cell response within the tumour tissue and peripheral blood of HPV+ HNSCC patients. In this way we will assess the frequency and function of these T cells (e.g. determining if some have a suppressive or “regulatory T cell” phenotype). HPV- HNSCC patients and healthy donors will be studied as controls.

2. **How are T cells recruited to this tumour tissue?** If a T cell-based therapy is to be effective for HPV+ HNSCC, the T cells must be capable of entering the tumour tissue. However, entry of circulating T lymphocytes into tissue is not a random process. It is regulated by interactions between chemokine receptors expressed on the T cell surface and chemokines expressed by the tumour tissue and associated blood vessels. Therefore to aid the development of effective T cell-based therapies for HNSCC, we shall determine the molecular mechanisms whereby T cells naturally infiltrate this tumour. Using multi-colour flow cytometry we will assess the expression of a panel of chemokine receptors on infiltrating T cell subsets. The results will be compared with T cells from paired blood samples to look for evidence of selective recruitment into tissue. Chemokine expression will be explored by immunohistochemistry on tumour sections (Figure 2). Functional testing of chemokine receptors on tumour-infiltrating T cells will be conducted using chemotaxis assays\textsuperscript{14,15}. 

121
Figure 2. Expression of the chemokine CCL4 on blood vessels (brown staining) within another virus-associated malignancy, Nasopharyngeal carcinoma. We recently demonstrated that this recruits T cells expressing the chemokine receptor CCR5.15

References


How are you planning to ensure adequate supervision?

The student will be fully supported through scheduled weekly meetings with the primary supervisor to discuss experiments and any concerns the student may have. The student will work closely with the primary supervisor and have regular (almost daily) access to both him and members of the Lee and Mehanna labs. Day to day the student will work alongside experienced postdoctoral and postgraduate researchers who will be working on other T cell-based projects.

The student role.

During the project, the student will spend most of their time engaged in laboratory-based research. Under the supervision of Dr Lee and following a period of training, they will design, conduct and interpret the results of experiments. They will also have time to read around the subject area, to understand the background to the project and to keep up to date with recent developments. The student will take an active role in weekly lab meetings/journal clubs in which they will have a chance to discuss their own data and critique the work of others.
Lead Supervisor: Dr. Daniel Tennant

Co Supervisor: Prof. David Blackbourn

Project Title: How do different cancer-causing viruses subvert the hypoxic response in cells?

Department: Cancer Sciences

Contact Email: d.tennant@bham.ac.uk

Is the project cancer related? Yes or No

Is the project cancer related?

Yes

No

Discipline:

Cancer Sciences

Immunology

Pathology

Anatomy

Metabolic Medicine

Endocrinology

Haematology

Liver & GI Medicine

Infection

Evolutionary biology in Clinical medicine

Project Outline

Certain viruses contribute to the development of ~15% of human cancers. Preventing or treating these infections therefore provides an opportunity to reduce the human cancer burden globally. However, in order to do so, we need to understand the biology of these oncogenic viruses. Here, we propose to study the pathogenesis of Kaposi’s sarcoma-associated herpesvirus (KSHV) in order to developing strategies to prevent its oncogenicity.

The growth of tumours is fundamentally regulated by their controlled and coordinated response to low oxygen (hypoxic) conditions. Evidence in this regard is particularly clear in those familial tumours that have mutations in the oxygen-sensing machinery, and as a result are highly angiogenic and treatment resistant. Kaposi’s sarcoma, much like the familial tumours described above, is characterised as a highly vascularised tumour, suggestive of de-regulated hypoxic signalling playing a role in its formation.

We have preliminary data to suggest that KSHV infection of cells results in a change in the hypoxic response. Here, we will investigate how KSHV regulates hypoxia, and vice versa. We hypothesise that KSHV subverts the oxygen sensing mechanisms of the cell, which facilitates tumour development.

This project will investigate the expression and activity of the hypoxia-regulated transcription factor, hypoxia-inducible factor (HIF) in KSHV-infected cells in both normal oxygen (normoxic) and hypoxic conditions. In addition, we will investigate if hypoxia augments KSHV infection and replication.
Techniques involved in this project include: virus culture and infection, mammalian cell culture, western blotting, quantitative PCR, ELISA.

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<tr>
<td>To ensure the student becomes familiar with and competent in the necessary laboratory techniques, careful hands-on supervision will be provided by both Dr. Tennant and Professor Blackbourn and members of their teams. As the student’s confidence and competence grows, s/he will work increasingly independently, but with the knowledge that expertise is available on demand.</td>
</tr>
<tr>
<td>The student and the two supervisors will meet weekly for progress reviews and laboratory group meetings will occur regularly. These meetings will provide opportunity for data to be presented and commented upon in order to ensure that the project remains on track. Dr. Tennant will help with hypoxia experiments. On a day-to-day basis, Debbie Smith in the Tennant lab is very experienced with the cell culture involved in this project, and both she and a number of PhD students can help with all other experiments.</td>
</tr>
<tr>
<td>Thus, the student will work alongside these other researchers in the Tennant and Blackbourn laboratories, becoming familiar with project specific techniques and gaining a wider appreciation of work on going in both laboratories.</td>
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<td>The student will be trained in all necessary techniques and will therefore perform the laboratory work required to deliver the project goals. Importantly, to ensure ownership of the project, the student will be given every opportunity to contribute intellectually to the direction of the project, through discussion with the supervisors during weekly review meetings and during laboratory group meetings. To aid this intellectual input and to facilitate writing the thesis, the student will be expected to review the literature underpinning the project and to keep abreast of the current literature enabling the development and direction of the project and ensuring it remains cutting edge.</td>
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</table>
**Project Outline**

Metformin, a biguaine derivative is the most widely prescribed drug to treat hyperglycaemia in patients with type II diabetes. Despite being introduced over 50 years ago, the exact mechanism of action remains to be fully elucidated. We have recently analysed the outcome of patients with type II diabetes undergoing isolated coronary artery bypass graft (CABG) surgery in the Queen Elizabeth Hospital over the past 7 years. Of the 773 patients identified, 423 patients were found to be taking metformin. We then investigated the risk of myocardial dysfunction (incidence of low cardiac output episodes) following surgery in patients on metformin versus those on other medications to control their diabetes. We found that patients on metformin were 40% less likely to exhibit a functional deficit post-surgery than those on other medications (17% vs 28% - p=0.0001).

We hypothesise that this protection from functional deficit is due to a protection of the heart tissue from ischaemia-reperfusion injury by metformin. Although the exact nature of the action of metformin is unclear, there is much evidence to suggest that metformin treatment leads to the activation of the energy sensor, AMP-activated protein kinase (AMPK). However, it is unlikely to directly activate AMPK as it does not influence the phosphorylation of AMPK in cell-free assay. Instead there is evidence that AMPK activation by Metformin is secondary to its mild and specific inhibition of complex I of the mitochondrial electron transport chain.

We have set up an *in vitro* model to investigate the mechanism by which metformin protects the heart from ischaemia-reperfusion (I-R) injury. Using this model the project will involve treating human primary cardiac myocytes with metformin and then simulating ischaemia-reperfusion by incubating in a low oxygen (0.1%) environment without any nutrients, followed by exposing them to 21% oxygen and normal nutrient conditions. The student will examine the effect of these conditions on AMPK activation, and test whether the activation of this signalling molecule is necessary for metformin-mediated protection from cell death. The student will also examine how the metabolism changes during I-R, and whether metformin results in a change in the metabolic response to I-R.
This project will use a number of different laboratory techniques, including tissue culture, western blotting, enzyme assays and metabolic tracer studies.

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The student and the two supervisors will meet weekly for progress reviews and laboratory group meetings will occur regularly. These meetings will provide opportunity for data to be presented and commented upon in order to ensure that the project remains on track. Dr. Tennant will help with hypoxia experiments. On a day-to-day basis, Debbie Smith in the Tennant lab is very experienced with the cell culture involved in this project, and both she and a number of PhD students can help with all other experiments.

Thus, the student will work alongside these other researchers in the Tennant laboratory, becoming familiar with project specific techniques and gaining a wider appreciation of work on going in both laboratories.

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<td>The student will be trained in all necessary techniques and will therefore perform the laboratory work required to deliver the project goals. Importantly, to ensure ownership of the project, the student will be given every opportunity to contribute intellectually to the direction of the project, through discussion with the supervisors during weekly review meetings and during laboratory group meetings. To aid this intellectual input and to facilitate writing the thesis, the student will be expected to review the literature underpinning the project and to keep abreast of the current literature enabling the development and direction of the project and ensuring it remains cutting edge.</td>
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Kaposi sarcoma-associated herpesvirus (KSHV) is associated with significant clinical problems, being the cause of the most frequent malignancy reported in HIV patients and sub-Saharan African men, namely Kaposi sarcoma. This virus causes a persistent infection in its human host which is normally controlled by the host T lymphocyte response, preventing disease from occurring. Here the virus lives in equilibrium with the host using mechanisms to minimize recognition and clearance by the immune response. However in some people, particularly those with impaired immune responses, the virus can infect cells and these can become malignancies of either endothelial or B lymphocyte origin. In these cases, the mechanisms the virus uses to avoid efficient T lymphocyte targeting become too effective and the virus-induced cancers are not controlled.

One mechanism KSHV uses is that it expresses a gene product called vFLIP that interferes with the apoptotic cell death program which is induced by T lymphocytes when they recognise virus infected cells. vFLIP is expressed in KSHV-associated malignancies and functions by either inhibiting apoptosis through interfering with caspase mediated killing, or by promoting cell survival by activating the NF-κB pathway.

In this project the student will explore the contribution of vFLIP to protection from T cell mediated clearance. Here the student will engineer the vFLIP gene into target cell lines and confirm the gene is expressed. Secondly they will ask whether CD4 or CD8 T lymphocytes, which recognise antigens expressed by the target cell, can kill or prevent the outgrowth of the vFLIP expressing cells i.e. does vFLIP expressed in isolation protect cells from being killed?

If vFLIP does not prevent killing, one of the other six genes expressed by the virus in the malignancies will be expressed in the target cells and protection from killing assessed. As we expect that vFLIP will have a protective effect, the student will determine whether adding inhibitors of the NF-κB pathway can restore T cell killing of these cells. Preliminary analysis suggests that at least one NF-κB inhibitor, azidothymidine, can restore sensitivity to CD4+ T cell killing. The student will further
explore this phenomenon and assess if AZT treatment restores CD8+ T cell killing of these targets.

Finally the student will ask if KSHV-specific T cells can kill KSHV-associated tumour cell lines when they have been treated with the azidothymidine. An understanding of how to make KSHV-associated tumours more sensitive to T cell mediated control opens the way for administering better therapies to patients with KSHV disease. Thus should treatment of tumours with azidothymidine allow better immune control, this will ultimately lead to the development of exciting clinical trials with a drug which has been extensively used in human populations and better patient outcomes.

**How are you planning to ensure adequate supervision?**

The student will be primarily supervised by Dr Hislop and embedded within his group in the School of Cancer Sciences. As well as Dr Hislop working on this project, a technician will also work in a related area on this project who, as well as Dr Hislop, can also monitor the students work day to day in the lab. The student will have weekly meetings with Dr Hislop to review experiments and data as well as plan new experiments and also meet with Prof Blackbourn at appropriate intervals.

**The student role.**

The student will perform the research outlined above. This will involve performing much tissue culture, including growing T lymphocyte clones and target cells. The student will assess T lymphocyte function using immunoassay techniques such as ELISA and flow cytometry. They will also analyse for gene and protein expression, using techniques such as western blot analysis.

The student will be embedded within wider groups whose interests include immune control of oncogenic herpesviruses and immunotherapy for tumours. They will be expected to go to group meetings, journal discussion meetings, departmental seminars (weekly) and Cancer Immunology and Immunotherapy Centre meetings (monthly).
**Lead Supervisor:** Prof Alan Rickinson

**Co Supervisor**
Dr Andrew Hislop

**Project Title:** Immune control of Epstein-Barr virus in patients with immunodeficiency syndromes

**Department:** Cancer Sciences

**Contact Email: Telephone:**
Prof Rickinson [a.b.rickinson@bham.ac.uk](mailto:a.b.rickinson@bham.ac.uk) 0121 414 4492
Dr Hislop [a.d.hislop@bham.ac.uk](mailto:a.d.hislop@bham.ac.uk) 0121 414 7983

**Is the project cancer related?** Yes

**Discipline:**

<table>
<thead>
<tr>
<th>Cancer Science</th>
<th>Pathology</th>
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<td>Liver &amp; GI Medicine</td>
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<td>Infection</td>
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<td>Evolutionary biology in Clinical medicine</td>
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**Project Outline**

Epstein-Barr virus (EBV) is a human herpes virus with potent oncogenic potential, being most frequently associated with Burkitt lymphoma, Hodgkin lymphoma, nasopharyngeal carcinoma and post transplant lymphoproliferative disease. Greater than ninety percent of the adult population is infected with this virus and, in general, suffer few ill effects. This is because the virus has evolved to live in equilibrium with the host, with the host immune response controlling the growth transforming potential of the virus and the virus having mechanisms to avoid immune mediated clearance, allowing it to persist. This virus host balance is mostly well maintained, however when the equilibrium is disrupted, disease can occur.

Good evidence exists for the cellular immune response being critical to the control of this virus and its associated malignancies, but it is less clear which component. One group of patients that can teach us much about immune control of this virus are those with primary immunodeficiencies. These people have defects in their immune system and in some cases poorly control, or respond inappropriately, to infection with the normally innocuous EBV. Indeed we have previously found that boys with a primary immunodeficiency, X-linked lymphoproliferative disease, have CD8 T cells that can not efficiently recognise and kill B lymphocytes, one of the main targets for EBV infection, explaining why this group of patients show such a high frequency of B cell malignancies (Hislop et al 2010).

In this project the student will examine B and T lymphocyte function from patients who have an X-linked disorder in which the gene X-linked inhibitor of apoptosis protein (XIAP) is disrupted. These patients show poor control of their EBV infection, with patients often presenting with haemophagocytic lymphohistiocytosis. Little is know however about the functional potential of their cellular immune response. Here the student will quantify the ex-vivo CD8+ and CD4+ T cell components of the cellular immune response to EBV in these patients and compare it to healthy donors who effectively control their persistent EBV-infection. The student will then establish EBV- and other virus-specific T cell clones from these patients and assess their function in vitro when challenged with EBV infected target cells and assess their growth potential. This will assess the T cell competence in these donors allowing us
to understand whether there is a T cell defect in the control of EBV in this setting.

The student will also examine the sensitivity of EBV infected B cells and T cells to apoptotic stimuli, to additionally understand the consequence of the loss of XIAP.

The results of this project will give a better understanding of the requirements of immune control of EBV infection in XIAP patients. Secondly it will give an indication of what therapeutic interventions may be considered in treating this disease.

**Hislop AD, Palendira U, Leese AM, Arkwright PD, Rohrlich PS, Tangye SG, Gaspar HB, Lankester AC, Moretta A, Rickinson AB. 2010. Impaired Epstein-Barr virus-specific CD8+ T cell function in X-linked lymphoproliferative disease is restricted to SLAM family positive B cell targets. Blood 116:3249-3257.**

**How are you planning to ensure adequate supervision?**

The student will be primarily supervised by Prof Rickinson and embedded within his “T cell” group in the School of Cancer Sciences. As well as Dr Hislop working on this project, members of the T cell group can also monitor the students work day to day in the lab. The student will have weekly meetings with Prof Rickinson and Dr Hislop to review experiments and data as well as plan new experiments.

**The student role.**

The student will perform the research outlined above. This will involve performing much tissue culture, including growing T lymphocyte clones and target cells. The student will assess T lymphocyte function using immunoassay techniques such as ELISpot, ELISA and flow cytometry. They will also analyse for gene and protein expression, using techniques such as western blot analysis.

The student will be embedded within wider groups whose interests include immune control of oncogenic herpesviruses and immunotherapy for tumours. They will be expected to go to group meetings, journal discussion meetings, departmental seminars (weekly) and Cancer Immunology and Immunotherapy Centre meetings (monthly).
Cellular immune therapies are showing significant promise in the fields of oncology and autoimmune diseases. Underpinning most types of cellular therapy is the need to manipulate cells, more specifically separate desirable cells from undesirable cells. This is important as undesirable cell populations may give rise to unwanted side effects for example Graft vs Host disease (GVHD) or reduce the benefit of the disease. Thus cell separation technologies will underpin many of future cellular therapies.

This project provides an opportunity to develop a new technology to purify and enrich cells once they have been grown in a clinically compatible manner. As a starting point we have modified columns, which have an excellent safety record for 'washing' human cells. We can modify these devices to capture specific cells and in this project will take the work the the next level. Experience in medical device development will be gained in addition to knowledge in antibody and peptide engineering (skills not taught in medical school!)

The project will culminate with the purification of antigen-specific cells from human blood and show that we can use these to attack tumour cells in vitro and possibly in vivo.

How are you planning to ensure adequate supervision?

Supervision will take place at two levels. Firstly, weekly laboratory group meetings are held in addition to supervision with Mark Cobbold and Oliver Goodyear. Secondly, day-to-day supervision will be provided by Oliver Goodyear to ensure the progression of the project.

The student role.

The role of the student will be to work closely alongside Dr Goodyear in developing the selection device in conjunction with polymer chemists and bioprocessing engineers. We have developed a miniaturised selection column that allows optimisation of cell capture and release parameters. Once these are optimised we will move to a large cell capture device.

Once the student is trained, they will be able to explore their own project relating to the selection of a particular cell type of their choosing. This could be for instance selecting regulatory T cells to prevent autoimmunity; or haematopoietic stem cells for stem cell transplantation; or even killer T cells for immunotherapy.
Project Title: Protein interaction studies with human PAPS synthases

Department: CEDAM

Contact Email: j.w.mueller@bham.ac.uk

Is the project cancer related? No

Discipline: Cancer Sciences Immunology
Pathology Anatomy
Metabolic Medicine Endocrinology
Haematology Liver & GI Medicine
Infection Evolutionary biology in
Clinical medicine

Project Outline

Sulfation of biomolecules including steroids is an essential process in the human body carried out by so-called sulfotransferases (SULTs). All mammalian SULTs require 3’-phospho-adenosine-5’-phosphosulfate (PAPS) for activity. PAPS is exclusively produced by two bi-functional PAPS synthase enzymes, PAPSS1 and PAPSS2. Compound heterozygous mutations in the gene encoding PAPSS2 have been shown to be associated with androgen excess (Noordam, Dhir et al., 2009). Lack of sulfation of the androgen precursor dehydroepiandrosterone (DHEA) to inactive DHEA sulfate (DHEAS) resulted in conversion of all available DHEA molecules toward active androgen, causing androgen excess. Similarly, a homozygous severe loss of function mutation in PAPSS2 has been shown to be associated with severe bone dysplasia (ul Haque et al., 2009; Ahmad et al., 1998) illustrating the crucial role of sulfation in bone and chondrocyte development. A major question resulting from these findings is why the other enzyme isoform (PAPSS1), which is ubiquitously expressed, could not compensate for this deficiency.

While analysing ligand binding and other biophysical properties of PAPSS1 and PAPSS2, it became apparent that the two proteins differ remarkably in their stability (van den Boom et al., 2012). While PAPSS1 seemed to be a “normal” enzyme, PAPSS2 was found to be partially unfolded, i.e. in a much less stable state, at body temperature. Moreover, PAPSS2 lost its enzymatic activity at 37 °C with half lives in the range of minutes under in vitro conditions. Next, it was found that the nucleotides involved in overall PAPS bio-synthesis tremendously influenced the stability of PAPS synthases, with the intermediate adenosine-5’-phosphosulfate (APS) having the most prominent effect on PAPS synthases: it increased the unfolding temperature (of at least part) of the enzymes by 16 °C and effectively prevented aggregation of these proteins. Currently, we think of PAPSS2 as a putative bio-sensor that responds to changing APS nucleotide levels by partial unfolding. In this regard we are keen to find transient protein interacting partners that either interact with PAPSS2 only at elevated temperatures or low nucleotide levels. Searching for such proteins, which may function as chaperones, is the central aim of this proposal.

To do so, state-of-the-art TAP tagging (tandem affinity purification) as well as mass
spectrometry-based identification of the pulled proteins is planned to be carried out. Once the procedure is established, various conditions are then to be compared – the set of interacting proteins should be compared at high vs low temperature as well as high vs low APS concentration. This will give important insights into PAPSS function within mammalian sulfation pathways, with important clinical implications for androgen and bone biology, but also for drug metabolism where sulfation is a key determinant.

Further readings (supervisors of this project):


**How are you planning to ensure adequate supervision?**

The student will receive daily supervision by Dr Mueller, during the initial phase working alongside him at the bench, with developing skills the student will be able to carry out defined elements of the research project autonomously with daily feedback.

In addition, the student will participate and present at the weekly lab meetings of the Arlt group and discuss his/her work in all detail with Prof Arlt and Dr Mueller in biweekly research meetings of the steroid sulfation sub-group.

**The student role.**

Firstly, the student will clone the TAP expression constructs of PAPSS2. He or she will then establish (with the aid of Dr Mueller) pull down conditions. Mass spectrometric analysis is primarily carried out specialist core facility staff, but the student will liaise with them and gain detailed insight into the methodologies, their pitfalls and opportunities. Data analysis and planning of further experimental procedures will be carried out by the student in close collaboration with Dr Mueller.
**Lead Supervisor:** Dr Rik Bryan

**Co Supervisor** Dr Ashley Martin, Dr Doug Ward and Dr Wenbin Wei

**Project Title:** Identification and Evaluation of Biomarkers for the Detection of Bladder Cancer

**Department:** Cancer Sciences

**Contact Email:** d.g.ward@bham.ac.uk

**Telephone:**

**Is the project cancer related?** Yes

**Discipline:**

- Cancer Sciences [ ]
- Immunology [x]
- Pathology [ ]
- Anatomy [ ]
- Metabolic Medicine [ ]
- Endocrinology [ ]
- Haematology [ ]
- Liver & GI Medicine [ ]
- Infection [ ]
- Evolutionary biology in Clinical medicine [ ]

**Project Outline**

Bladder cancer is the 5th most common cancer in Western societies, responsible for 10,000 new cases and 5,000 deaths annually in the UK. The majority of patients present with non-muscle-invasive disease (NMIBC, stages Ta/T1/Tis); although non-life-threatening in most cases, NMIBC requires lifelong surveillance with flexible cystoscopy (an endoscopic bladder inspection) and urine cytology. Establishing accurate diagnostic (and prognostic) urinary biomarkers could significantly improve the quality of life for the 35-37,000 NMIBC patients undergoing surveillance each year.

This project aims to develop a panel of biomarkers to generate a urine test to detect bladder cancer. We are using proteomics (LC-MS/MS) to identify proteins at altered levels in the urine of patients with bladder cancer and also proteins secreted by bladder cancer cells cultured *in vitro*. Both approaches have generated candidate biomarkers, some of which we have shown to be significantly increased in the urine of patients presenting with primary bladder tumours. We are also analysing several DNA methylation markers in urine sediments which are look very promising for bladder cancer detection. This is currently being performed using the Massarray platform but we will soon be moving to next generation sequencing.

Within the area of ‘bladder cancer biomarkers’ an intercalated student may undertake research in:

1) Proteomic analysis of cell line ‘sheddomes’
2) Proteomic analysis of protein acetylation and glycosylation
3) Validation of candidate protein and DNA biomarkers in clinical samples for the detection, staging and monitoring of bladder cancer.
4)
### How are you planning to ensure adequate supervision?

The student will be supervised by Drs Ashley Martin and Doug Ward during all wet lab work. Dr Rik Bryan will provide additional expertise in bladder cancer, especially from the clinical perspective, and access to clinical samples. Dr Wenbin Wei will provide bioinformatic support. The student will meet with all four supervisors on a fortnightly basis.

### The student role.

This project will provide training in the discovery, validation and application of biomarkers using ‘omic’ and bioinformatic approaches. The project is mostly lab based and will provide an introduction to proteomics and epigenetics, both with the goal of biomarker discovery. The project may focus more on one approach than the other depending on the student’s interests and results obtained.
Graft versus Host disease (GVHD) is a major complication following allogeneic haematopoietic stem cell transplantation (SCT) and is associated with significant morbidity and mortality, affecting up to 40% of patients. Donor cells recognise the patient tissue as foreign and initiate a damaging immune response. Clinical evidence suggests that early intervention significantly improves patient outcome. University Hospitals Birmingham is the second largest UK transplant centre treating approximately 100 patients per year. Our ongoing collaboration with the medical team allows us access to clinical samples and outcome data from the majority of patients undergoing SCT; this puts us in a unique position to effectively study this disease.

Our work has shown that measuring cellular immune reconstitution shortly after transplant allows us to predict which patients will go on to develop GVHD (figure 1). To date our work has focussed on early T cell reconstitution dynamics post SCT. However, other leukocyte populations including B cells and antigen presenting cells have also been linked to the development of GVHD and this project aims to define the early reconstitution kinetics of these leukocyte populations. PBMC will be isolated post transplant and will be analysed by flow cytometry to determine B cell, T cell and dendritic cell numbers and phenotype. It will then be important to determine how these populations interact. Within the Moss group we have our own Imagestream cytometer (one of only 6 in the UK). As part of this project, we will use this cutting edge technology to study these cellular interactions in detail.

Other studies to date have focussed on the timepoint at which GVHD develops. Our work aims to identify patients who would benefit from early intervention strategies to prevent development of disease. This will improve morbidity and mortality associated with transplant, increase the numbers of patients eligible for transplant and improve the patient’s long term quality of life.

Figure 1. A logistic regression model based on the numbers of circulating T-cells in the early period after transplant has been developed. Based on the model,
the predicted probability of developing GvHD is shown on the x axis. The patients who actually went on to develop GVHD are represented in red whereas disease-free patients are in green. The model allows us to predict which patients will get GvHD with a sensitivity of 85% and a specificity of 100%.

How are you planning to ensure adequate supervision?

The Transplant group are a sub group of Prof Paul Moss’ lab led by Dr Croudace and Dr Inman with 3 PhD students (one of which is a medical graduate) and a clinical fellow. The group hold weekly Monday morning meetings to discuss progression of each of the transplant team’s projects. In addition, short presentations on key immunology topics are given during this meeting to ensure that the students have a good grounding in fundamental immunology. The larger Moss group meets on a Friday morning: one member of the group presents their work and following this there is a journal club. In addition, formal one-to-one meetings will be set up on a monthly basis, however, both Dr Croudace and Inman are present in the lab on a daily basis.

The student role.

- Attendance at weekly transplant group and Moss group meetings.
- Become proficient in both flow cytometry and image stream analysis
- Maintain lab book by keeping a clear account of every experiment
- Arrange and collect patient samples
- Communicate with clinical staff
- Learn basic data handling and analysis
- Present findings at weekly transplant meetings and at the Moss group meeting at the end of the project
- Learn to work independently after initial training
**Lead Supervisor:** Dr. Christian Ludwig

**Co Supervisor** Dr. Daniel Tennant

**Project Title:** Metabolic changes in OE33 cells under hypoxia and 5-FU treatment

**Department:** School of Cancer Sciences

**Contact Email:** C.Ludwig@bham.ac.uk  
**Telephone:** ext. 48362

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Is the project cancer related? **Yes**

**Discipline:**

- Cancer Sciences
- Immunology
- Pathology
- Anatomy
- Metabolic Medicine
- Endocrinology
- Haematology
- Liver & GI Medicine
- Infection
- Evolutionary biology in Clinical medicine

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**Project Outline**

In 1924 Otto Warburg postulated that cancer is a disease with metabolic origins, in which cells produce energy mainly from glucose. He further postulated that irreversible mitochondrial damage led to this phenotype in all cancer cells. While it is not likely that this is true, cancer cells often face challenging environments due to the rate of tumour growth, putting considerable strain on their metabolism. Therefore, cancer cells have to alter their metabolic strategy to survive under these hostile circumstances.

A large number of the most frequently used chemotherapies are known to alter cell metabolism. However, these drugs also target many areas of normal cell metabolism, leading to unpleasant side effects. Due to our lack of knowledge of the precise nature of the changes in cancer cell metabolism, we are, at present unable to design drugs with better specificity against the tumour. We therefore urgently need studies that investigate the specific changes in cancer cell metabolism.

Metabolomics approaches are increasingly used to understand cell metabolism. These approaches are able to provide a powerful insight into disease phenotypes, and in turn, suggest novel therapeutic approaches for disease control. However, a more targeted approach, introducing isotopically enriched metabolic precursors such as glucose and glutamine with isotopes which only occur at very low levels naturally, opens up an entirely new set of possible applications as specific metabolic pathways can be addressed and hence studied in much more detail. Depending on the metabolic pathway under consideration, different metabolic precursors have to be used.

Here we propose an 8 month project to study the effect of hypoxia and 5-FU (5-Fluorouracil) on oesophageal cancer cells OE33. During the project the student will cultivate these cells under different conditions and use 13C- & 15N-labeled metabolic precursors such as uniformly enriched 13C,15N-Arg, 15N-Gly and 13C-Met. The cultivated cells then will be extracted and subsequently analysed by nuclear magnetic resonance spectroscopy (NMR). The resulting NMR spectra will be analysed by our own published software, which will help to elucidate the underlying active metabolic pathways and ratios of label incorporation into the metabolites leading to creatine and phosphocreatine.
**How are you planning to ensure adequate supervision?**

The student and the two supervisors will meet weekly for progress reviews and laboratory group meetings will occur regularly. These meetings will provide opportunity for data to be presented and commented upon in order to ensure that the project remains on track.

Dr. Tennant is an expert in metabolism and runs his own lab and is currently supervising 4 PhD students who are involved in growing cell cultures and performing cell extraction procedures. Together with his technician Deborah Smith he will supervise the cultivation and extraction of the cell samples.

Dr. Ludwig's expertise is NMR spectroscopy. He is part of the Henry Wellcome Building for Biomolecular NMR spectroscopy and runs several NMR theory courses. He also currently supervises 1 PhD student and trains most new users to the NMR facility. He also wrote the software which will be used to analyse the NMR spectra.

**The student role.**

The student will be trained in all necessary techniques and will therefore perform the laboratory work required to deliver the project goals.

The student will grow cells using labelled metabolic precursors, extract the cell samples and analyse the NMR spectra. The student will be supervised during all procedures until they are familiar with the techniques and to help troubleshoot when problems occur. If the student wants to know more about NMR spectroscopy (e.g. how to run experiments etc), he/she will be given additional training, but this is not essential for this project.
Colorectal cancer (CRC) is the third most common cancer, and the second most common cause of cancer death, in the UK. The 5-year survival for CRC remains at around 50%, due to difficulties in treating advanced disease. Unfortunately, many cases are already advanced upon diagnosis. Recent developments in this field, such as the addition of novel targeted agents, have achieved only limited success. Thus, there remains an urgent need for an effective, systemic therapy.

There is compelling evidence that the immune response plays a central role in controlling CRC (Gallon et al., 2006, Tosolini et al., 2011). However, the tumour antigens that the lymphocytes are recognising remain undefined. Cytotoxic T lymphocytes (CTL) recognise peptidic fragments of antigen (termed epitopes) bound to MHC class-I molecules displayed on the cell surface. We have recently identified a number of tumour-associated posttranslationally modified epitopes displayed on the surface of tumour, but not normal tissue, from CRC patient samples. The modifications on these epitopes are the phosphorylation of serine or threonine residues. In cancers, many proteins are aberrantly phosphorylated; signalling is increased, allowing the cells to survive and proliferate. Thus, we suspect that these phosphopeptides are a key immunological signature of transformed self; a mechanism whereby cancer cells can be specifically targeted, as different from normal tissue, by CTL (Zarling et al., 2006).

The student selecting this project will investigate patient responses to these key, novel CRC-associated phosphopeptides that have been identified. This will involve using a variety of techniques, including: blood and tissue preparation, flow-cytometry, cell selection, cell culture, ELISpot, and killing assays. The aim of the wider project is to select CTL clones that are suitable for progressing into cancer vaccine strategies.

References

**How are you planning to ensure adequate supervision?**
The student will be supervised in the laboratory, on a day-to-day basis, by Sarah Penny. There is a laboratory technician, working on a similar project, who will be able to offer practical support. There will also be weekly meetings with Mark Cobbold and Sarah Penny to discuss the student’s work. In addition, weekly laboratory group meetings will be a forum to share ideas with a wider audience. These meetings are also a great opportunity to learn about other aspects of tumour immunology from this large group, with varied interests.

**The student role.**
The role of the student will entail working closely alongside Sarah Penny in investigating patients’ immune responses to these novel phosphopeptide epitopes. Much of their time will be spent in the laboratory, conducting immunoassays. These will reveal which of the phosphopeptide epitopes are recognised on many patient tumours, and thus would be good targets for future immunotherapeutic strategies. The student will then go on to grow CTL specific for these phosphopeptide targets and test these cells in numerous assays, including the killing of tumour cells. Previous students in our laboratory have been very successful, for example, presenting at international conferences and being named on key publications.
Lead Supervisor: Dr Graham S Taylor

Co Supervisor: Dr Heather Long

Project Title: Improving cancer immunotherapy by harnessing autophagy.

Department: School of Cancer Sciences

Contact Email: g.s.taylor@bham.ac.uk
Telephone: 0121 414 7983

Is the project cancer related? Yes

Discipline:
- Cancer Sciences
- Immunology
- Pathology
- Anatomy
- Metabolic Medicine
- Endocrinology
- Haematology
- Liver & GI Medicine
- Infection
- Evolutionary biology in Clinical medicine

Project Outline

Autophagy is a recently identified degradative pathway that is now known to play a key role in many cellular processes including cancer and immunity.

We have recently shown that autophagy can generate some, but not all of the immune epitopes from a tumour antigen that are recognised by immune T cells. Understanding why some epitopes are processed by autophagy while others are not is important for the development of effective cancer immunotherapies. You will contribute to this exciting area of research by exploring the mechanisms underpinning the selective generation of immune epitopes.

First, you will extend our original results\textsuperscript{1,2} into new areas, making use of a wide panel of tumour-specific T cell clones.

Second, you will compare the biochemical properties of the autophagy-dependent and independent immune epitopes using our newly developed assays.

Third, you will make T cell clones able to specifically recognise tumour cells to broaden the range of immune epitopes that are tested for autophagic processing.

In summary, you will gain experience of a range of immunological techniques and contribute to an important and exciting area of research.

1. Leung, Haigh, Mackay, Rickinson and Taylor. 2010 Nuclear location of an endogenously expressed antigen, EBNA1, restricts access to macroautophagy and the range of CD4 epitope display. \textit{PNAS} \textbf{107}, 2165-70.

How are you planning to ensure adequate supervision?
This project will be supervised by Dr Graham Taylor who will teach the student the required techniques and be available to meet on a daily basis. Dr Taylor has expertise in T cell immunology and autophagy and has a long-term interest in this research area. The student will also be supported by Dr Heather Long who has a direct interest in this work.

**The student role.**

The student will be able to commence work immediately, making use of existing T cells that have already been generated. Initially they will make use of well-established immune assays to build a broader picture of which tumour antigen epitopes are generated by autophagy and which are not. They will then examine the biochemistry of the different epitopes, focusing in particular on the susceptibility of the epitopes to degradative proteases. A collaborating research laboratory supplies these proteases. The student will also learn how to make new T cells in order to expand the work to a wide range of immune epitopes.

The student will become proficient in cellular immunology techniques (flow cytometry, cell culture, ELISAs). Their background knowledge of cancer immunology will be developed by attending laboratory meetings, journal clubs, and seminars given by visiting speakers.
**Project Outline**

Metformin is a safe, well tolerated oral hypoglycaemic drug widely used in Diabetes. Recent epidemiological, preclinical and clinical data suggest that metformin may have a therapeutic role in cancer. Metformin exhibits both direct and indirect activity against cancer. Indirect activity by the ability of metformin to lower circulating insulin which may be particularly important for the treatment of cancers associated with hyperinsulinemia and obesity (breast/colorectal/uterine). Metformin also exhibits direct inhibitory effects on cancer cells by activating AMP kinase, inhibiting mammalian target of rapamycin (mTOR) signaling and protein synthesis. (Dowling et al., 2012) Specifically in ovarian cancer, recent papers demonstrate that metformin augments cancer cell apoptosis in cell lines treated with platinum, inhibits cancer stem cells, reduces metastases and vascular counts in xenograft models. (Gotlieb et al., 2008; Rattan et al., 2011; Shank et al., 2012; Yasmeen et al., 2011). A Phase III trial of adjuvant metformin in breast cancer is in progress in Canada. Some phase II trials in prostate, colorectal, breast, uterine and lung cancer have been proposed/commenced.
Epithelial ovarian cancer has a poor 40% 5 year survival rate and low cost, well tolerated therapies to improve treatment at relapse are urgently needed. Patients with recurrent cancer frequently require tapping of ascites.

Main objectives of study

To identify potential biomarkers of metformin response in primary cultures of ovarian cancer cells derived from ascitic fluid after treatment with physiological doses of metformin.

Experimental work needed

1. Establish primary cultures from ascites tap from women with ovarian cancer (Under existing ethics at HBRC).
2. Demonstrate insulin sensitizing changes in these cell cultures after treatment with metformin using western blotting with phospho specific antibodies e.g. serine 473 of akt/PKB , Insulin receptor, IRS1 and 2. Expression of OCT1 (SLC22A1) – ‘metformin transporter’ and those related to the subunits of AMPK (PRKAA1, PRKAA2, PRKAG2) to demonstrate signalling on metformin. Expression of LKB1 and TSC2

The applicant will be based in Jeremy Tomlinson’s laboratory. Work from this project will inform a grant application for a Phase 2 trial of metformin in ovarian cancer. All patient samples will be collected using the pathways already in place with the University of Birmingham Human biorepository.

References
cancer. Gynecol Oncol 110, 246-250.

How are you planning to ensure adequate supervision?

The student will be fully supported through scheduled weekly meetings with the supervisors to discuss experiments and any concerns the student may have. The student will work closely with the supervisors and have regular access to other staff at the labs. Day to day the student will work alongside other experienced researchers. The techniques for primary cultures and Immunohistochemistry are well established.

The student role.

During the project, the student will spend most of their time engaged in laboratory-based research. Under supervision and following a period of training, they will design, conduct and interpret the results of experiments. They will also have time to read around the subject area, to understand the background to the project and to keep up to date with recent developments. The student will take an active role in weekly lab meetings/journal clubs in which they will have a chance to discuss their own data and critique the work of others.
| Lead Supervisor: | Dr. Maarten Hoogenkamp |
| Co Supervisor | Dr. Vesna Stanulovic |
| Project Title: | The role of LMO2 overexpression in acute myeloid leukaemia |
| Department: | School of Cancer Sciences |
| Contact Email: Telephone: | 0121 4143837 |

| Is the project cancer related? | Yes |
| Discipline: | Cancer Sciences | Immunology |
| | Pathology | Anatomy |
| | Metabolic Medicine | Endocrinology |
| | Haematology | Liver & GI Medicine |
| | Infection | Evolutionary biology in Clinical medicine |

**Project Outline**

Aberrant regulation of transcription factors is a common causative factor of cancer development. The LIM-domain protein LMO2 is an established T-cell oncogenic protein and is over-expressed in over 60% of T-ALLs. Chromosomal abnormalities of *lmo2* have been found in approximately 8% of paediatric T-ALL cases, with a mortality of around 20%

LMO2 is a protein that does not bind DNA directly, but is a component of particular DNA binding complexes. The best described complex is within the erythroid lineage, where LMO2 bridges between the DNA binding factors Tal1/E2A and GATA-1 together with its co-factor Ldb-1. In T-ALL GATA-1 is not present and a second Tal1/E2A dimer may take its place there.

Besides the role of LMO2 in T-ALL, LMO2 is also over-expressed in a significant proportion of acute myeloid leukaemia (AML). However, little is known about the consequences of LMO2 over-expression in non-T-cell lineages. To address this we have generated ES cells expressing a doxycycline inducible LMO2 (iLMO2) on a further wild-type background. Using an established culture system, we can differentiate ES cells in vitro into defined developmental stages, including myeloid progenitors. We will use this system to generate myeloid progenitors from the iLMO2 cell line. LMO2 will be induced at high levels and the effect of this induction on cell differentiation and gene expression will be measured, comparing them to non-induced cultures. At any stage, the over expression of LMO2 can be removed by washing out doxycycline from the medium, thus returning the cells to the wild type phenotype.

We will control these experiments by measuring LMO2 expression before and after induction at the mRNA and protein level, and assess the cellular localisation by microscopy. We will then establish the effects of high LMO2 expression on myeloid differentiation by monitoring morphology, detection of surface markers, and measuring the expression of genes known to be involved in macrophage development.
In addition to the analysis of the iLMO2 ES cell line, we will also aim to create new inducible ES cell lines. We have a parental cell line into which protein coding sequences can be introduced from a plasmid via CRE mediated recombination, which makes this a highly efficient method of targeting. We will do this for the aforementioned Ldb-1, which is crucial for LMO2 function, and for proteins identified in the initial body of work to be possibly involved in LMO2-induced alterations to myeloid development.

Techniques to be used in this project:
Cell culture / ES cell differentiation; Western blotting; Microscopy; Flow cytometry; cDNA synthesis; Quantitative PCR; DNA Cloning

How are you planning to ensure adequate supervision?
Both listed supervisors spend a significant proportion of their time at the bench in the laboratory, are skilled in the techniques mentioned above, and have prior experience in supervision of staff and students. This ensures that supervision and support is available on a daily basis throughout the eight months project. Every technique will initially be performed together with a supervisor, hopefully resulting in more independence later into the project.

In addition there will be a weekly meeting to overview and discuss the progress and next steps, or problems that have been encountered. A weekly journal club will be held in which current literature on this and related topics will be discussed.

The student role.
The student is expected to be motivated and to actively participate in the research group. The student will be primarily responsible for performing the experiments and interpreting the results of the above project, albeit in close association with the supervisors.

The student should be motivated to learn the different techniques, which are established protocols in the lab. We will work towards the student becoming more independent over the eight months in both the practical aspects of the project as well as in the ability to interpret the obtained data.
Lead Supervisor:  Dr Peter Searle

Co Supervisor  Dr Grant Stewart  
(and potentially Dr Farhat Khanim)

Project Title: Investigating the DNA damage response and possible drug interactions with prodrug activation gene therapy using nitroreductase and CB1954.

Department: Cancer Sciences

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Telephone:  0121 414 4487

Is the project cancer related?  Yes

Project Outline

The bacterial enzyme nitroreductase catalyses the conversion of a relatively non-toxic prodrug CB1954 into a bi-functional alkylating agent that introduces interstrand crosslinks in DNA, resulting in cytotoxicity. We are developing a “gene therapy” treatment for locally recurrent prostate cancer, in which a replication-defective adenovirus encoding nitroreductase is injected into the prostate, and the patient is then treated with the prodrug. Localised production of nitroreductase in the cancerous prostate is expected to activate the CB1954, killing the cancer cells. Initial clinical trials suggest this could reduce the tumour burden and delay progression of the disease in some patients. This project aims to understand more about how the cells respond to the DNA damage caused by the prodrug activation therapy, and investigate whether other drugs already used for a variety of clinical indications could enhance the therapeutic window.

There are two aspects to this project; firstly, we aim to investigate whether drugs that are already licensed for other applications could synergise with the nitroreductase/CB1954 treatment, to enhance the cytotoxicity against prostate cancer cell lines in vitro. This will exploit a panel of ~96 drugs that has been assembled by Dr Farhat Khanim (Biosciences). Secondly, we aim to investigate the nature and kinetics of the DNA damage response induced by nitroreductase/CB1954, including immunofluorescent analysis of histone H2AX as a marker of crosslink-induced double-strand breaks in DNA. If any drugs are found to synergise with nitroreductase/CB1954 for inducing cytotoxicity, the project will investigate whether they alter the amount of DNA damage, or the kinetics of its repair.

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<thead>
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<th>How are you planning to ensure adequate supervision?</th>
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<td>Dr Searle will train the student in cell culture, and to perform the cytotoxicity assays using nitroreductase/CB1954 and the additional panel of drugs. Dr Stewart or suitable postdocs/PhD students in his group will provide training in the immunofluorescence and western blot methods to analyse the DNA damage response. Dr Searle will meet with the student at least once per week to discuss results and future experiments, jointly with Dr Stewart when appropriate.</td>
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<tr>
<th>The student role.</th>
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<td>This is a laboratory-based research project, involving a variety of widely applicable research methods including cell culture, cytotoxicity assays, working with genetically modified virus vectors, immunofluorescence, and western blotting. It will also require background reading particularly regarding prostate cancer and its treatment, gene therapy, any promising drugs from the panel, and the DNA damage response. The student will initially be trained by workers experienced in each of the techniques, but will be expected after an initial period of close supervision to be able to work relatively independently. Throughout the project, the student will be expected to take an increasing role in the design and interpretation of their experiments.</td>
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Nitric oxide (NO) plays an important role in cardiovascular homeostasis. Recently, there has been a growing appreciation that nitrite and nitrate can be chemically reduced \textit{in vivo} to biologically active nitric oxide (NO) under the conditions of hypoxia and acidosis. Nitrite can elicit vasodilatation, reduce blood pressure, protect against myocardial ischemia-reperfusion injury, and modulate exercise efficiency. Further, it has been reported that nitrate also mediates these effects via conversion to nitrite in the oral cavity by commensal bacteria containing nitrate reductase enzyme systems.

Our research group and others have demonstrated in healthy volunteers that exogenous administration of nitrite functions as a more potent vasodilator in the human circulation. In addition, we have recently demonstrated that systemic nitrite infusion reduces right atrial pressure and pulmonary vascular resistance with relatively modest reductions in systemic vascular resistance and mean arterial blood pressure in patients with severe heart failure. However despite these novel findings, the precise mechanism(s) of nitrite-mediated vasorelaxation still remains unclear.

Several mechanisms of nitrite bioconversion to NO have been proposed; these include endothelial nitric oxide synthase, myoglobin, haemoglobin and aldehyde dehydrogenase. However, the precise downstream mechanism of nitrite-mediated vasorelaxation still remains unclear. \textit{Therefore, the main objective of this project will be to elucidate the downstream mechanism of nitrite in isolated blood vessels from mice.}

To determine the mechanism of nitrite in vasorelaxation, this project will use a broad spectrum of techniques to assess:

(1) conduit and resistance vascular function by using various pharmacological inhibitors on the myograph system (a system that measures contractility and vasodilatation in isolated blood vessels).

(2) the downstream mechanism(s) of nitrite-mediated pathways by Western blotting.
### How are you planning to ensure adequate supervision?

Supervision will be provided at all times during the project by Dr Madhani.

Thoracic aorta and mesenteric vessels will be provided to the student and supervision in how to do myography and molecular assessment will be provided.

### The student role.

The student role will be to work as a team in the research group and to provide pharmacological and molecular evidence to the nitrite-mediated vasorelaxtion. The aim of this research project will be to provide basic science evidence, which will complement our recent findings in heart failure patients.
Persistent perineal pain is one of the most commonly experienced health problems associated with vaginal birth. It is a symptom highly related to perineal trauma and can impact on a woman’s physical and psychological well-being as well as her relationship with her baby and family. Around 85% of women who have a vaginal delivery will sustain perineal trauma, which occurs either spontaneously or as a consequence of an episiotomy, and three-quarters of women will require suturing to facilitate healing of the disrupted tissue. Studies investigating maternal morbidity have reported that for some women, perineal pain persists well beyond the postnatal period.

Childbirth related perineal trauma can have a major adverse impact on women’s health both at the short and long term. Therefore, improved techniques of managing the perineum intrapartum and immediately postnatal can have a significant impact on women’s health. In current clinical practice. There is a wide variation in practice between different countries in Europe and even between different units within the same country. Moreover, there are several intrapartum interventions that are being practiced to reduce the risk of trauma, albeit, not based on strong evidence.

The aim of this project is to use systematic review methodology to assess the therapeutic effectiveness of the different proposed isolated and combined interventions in reducing the risk of childbirth related perineal trauma. Information from this project will help in developing a proposed model of best practice that can then be tested in appropriately designed clinical trials. The clinical trials will be undertaken as a separate project but will be dependent on the findings of these reviews.
**How are you planning to ensure adequate supervision?**

Both the lead supervisor and the co-supervisor have a long-standing experience in conducting systematic reviews for therapeutic evaluation and diagnostic accuracy. Both supervisors work in the same department. The lead supervisor is a clinical expert in the field and a co-author on several Cochrane reviews related to the subject matter.

**The student role.**

The student will co-ordinate the project and will be actively involved, under guidance from the supervisor, with the undertaking of the above reviews. This project will be a great opportunity for students to gain experience in undertaking health care related evidence synthesis methodology. We envisage that output from this project will lead to several publications in high impact journals. Moreover, the information generated will provide essential supplementary information for future grant applications and clinical trials.
Myeloma is a cancer of bone marrow plasma cells that impairs normal haemopoiesis and antibody production, destroys the skeleton and by secretion of M-protein causes renal failure. Treatment with high dose cytotoxic drugs and stem cell rescue improves survival. More recently biological therapies including thalidomide and its analogues and proteasome inhibitors have provided further improvement in survival but at a pharmaceutical cost of up to £50,000 per patient per year. Cures remain elusive and the disease kills 3,000 people per year in the UK.

Our translational research aims to find new uses for old drugs. The potential of this approach has already been dramatically proven in case of thalidomide which has improved response rates in patients over 65 and extended median survival by up to 18 months compared to standard melphalan and prednisone therapy. To date our own drug redeployment strategies have focused on other malignancies including acute myeloid leukaemia chronic lymphocytic leukaemia and Burkitts lymphoma and have lead to clinical trials both in the UK (ISRCTN50635541 & ISRCTN99131400) and in sub-Saharan Africa (ISRCTN34303497). However, we are now seeking new drug redeployment opportunities in Myeloma.

We have identified that the anti-helminthic niclosamide has anti-myeloma activity (Khanim FL et al. Blood Cancer J. 2011 Oct;1 (10):e39). We have also now identified that myeloma cell killing by niclosamide is enhanced by combining it with the antiepileptic drug sodium valproate.

This project will endeavour to determine how valproate works in combination with niclosamide. We have already identified that valproate regulates the the acetylation and thereby activity of superoxide dismutase, an enzyme important in protecting cells against internal attack from reactive oxygen species. Our current Intercalating BMedSc student (Lauren Ferrotti) is seeking to identify other proteins regulated by Valproate in this way using proteomic approaches. Next years project will be to perform experiments that reveal how and which of these newly identified targets contribute to the enhanced killing of myeloma cells by the combination of niclosamide and valproate.
In addition the student will assist in collaborative experiments with the University of Sheffield in which xenograft models of Myeloma will be used to determine the activity of niclosamide and valproate against myeloma cells \textit{in vivo} as well as their impact on M-protein levels and bone disease.

\textbf{How are you planning to ensure adequate supervision?}

We have taken previous Intercalating BMedSc on this type of project; Hannah Giles (2009/2010); Blair Merrick (2010/2011); Susan Raffles (2011/2012); Lauren Ferretti (2012/2013). The projects of HG and BM were highly successful and both were authors on the Niclosamide paper. SR came top in her year, won the Claire Ripley Prize and the Doctors Academy – University of Dundee Award. A manuscript is in preparation that has both SR and LF as authors. The day to day supervision of these students was provided by Farhat Khanim who works closely with the students, with support from the wider Bunce/Drayson team. Regular meetings were held with both Prof Drayson and Professor Bunce together and individually. The same form of supervision will be adopted for next year’s project. Our aim is to provide students with a positive experience of translational cancer research and understanding of the impact that their research can have on developments in the clinic. This is why we endeavour to train the students to the best of our ability so that they will become the clinical academics of the future.

\textbf{The student role.}

The student will perform wet lab based experiments in the School of Biosciences. This will include training in tissue culture, molecular biology approaches, cell analyses by flow cytometry and basic biochemical analyses including western blotting. The student will become an integrated member of the wider research team for the duration of the study. By the end the student will be expected to have real intellectual input to the study including research direction and experimental design. We assume no prior knowledge or experience and will teach everything the student needs during their project. The only thing the student needs to bring with them is enthusiasm, motivation and a desire to learn.
Lead Supervisor: Farhat Khanim

Co Supervisor: Mark Webber/ Chris Bunce

Project Title: Regulation of AML cell survival by bacterial derived nucleotide diphosphate kinase (NDK)

Department: Biosciences
Division of Immunity and Infection

Contact Email: F.L.Khanim@bham.ac.uk
Telephone: 48680

Is the project cancer related? Yes

Is the project cancer related? Yes

Discipline: Cancer Sciences
Pathology
Metabolic Medicine
Haematology
Infection

Immunoology
Anatomy
Endocrinology
Liver & GI Medicine
Evolutionary biology in Clinical medicine

Project Outline

Acute myeloid leukaemia (AML) is an aggressive cancer where tumour cells rapidly infiltrate the bone marrow and blood, creating deficits in red cells, platelets and immune cells. If untreated, patients die within weeks of diagnosis. Current therapies involve high grade chemotherapy, which require antibiotics and red cell and platelet transfusions to compensate for treatment-induced damage to the hematopoietic system. However, 60-70 % of patients are over the age of 60 and unable to tolerate these therapies. Survival in this cohort remains dismal. Many of these older patients develop from a previous myelodysplastic syndrome (MDS). Both MDS and AML patients are prone to recurrent bacterial infections which often lead to the cause of death. However, we hypothesise that these infections may also be drivers of the transition from MDS to AML and progression of the acute phase of disease.

We have shown that a human nucleotide diphosphate kinase (NDK) called Nm23-H1 promotes the survival of acute myeloid leukaemia (AML) cells via a sophisticated positive feedback mechanism that drives the expansion of the tumour. First AML Stem cells secrete Nm23-H1 which then binds to more mature cells in the tumour clone causing their increased survival. In addition binding of Nm23-H1 by the mature cells induces them to secrete cytokines that in turn act upon the AML stem cells to also promote their survival and continued proliferation. However, the NDK family are a very highly conserved family of proteins and many of the bacteria that cause infections in AML and MDS patients secrete structurally similar NDKs.

This project will determine if bacterial derived NDKs can recapitulate and/or augment the actions of Nm23-H1 in MDS and AML. The experiments will involve the generation of recombinant proteins, their exposure to AML cells, the analysis of what cells bind the protein, what they secrete in response to the protein and the ability of those secreted signals to affect the biology of leukaemic and normal haemopoietic stem cells.
How are you planning to ensure adequate supervision?

We have taken previous Intercalating BMedSc on this type of project; Hannah Giles (2009/2010); Blair Merrick (2010/2011); Susan Raffles (2011/2012); Lauren Ferrotti (2012/2013). The projects of HG and BM were highly successful and both were authors on a published manuscript. SR came top in her year, won the Claire Ripley Prize and the Doctors Academy – University of Dundee Award. A manuscript is in preparation that has both SR and LF as authors. The day to day supervision of these students was provided by Farhat Khanim with support from the whole Bunce/Drayson team. The same form of supervision will be adopted for this project. However, the student will benefit from further supervision from Dr Webber and his team. In so doing the Student will learn from internationally competitive researchers in both the fields of cancer biology and infection. Our aim is to provide students with a positive experience of translational cancer research and understanding of the impact that their research can have on developments in the clinic. This is why we endeavour to train the students to the best of our ability so that they will become the clinical academics of the future.

The student role.

The student will perform wet lab based experiments in the School of Biosciences. This will include training in tissue culture, molecular biology approaches, cell analyses by flow cytometry and basic biochemical analyses including western blotting. The student will become an integrated member of the wider research team for the duration of the study. By the end the student will be expected to have real intellectual input to the study including research direction and experimental design. We assume no prior knowledge or experience and will teach everything the student needs during their project. The only thing the student needs to bring with them is enthusiasm, motivation and a desire to learn.
The global incidence of all-cancer cases is expected to increase from 12.7 million new cases in 2008 to 22.2 million by 2030, the majority will occur in under resourced countries. There is an urgent need to identify low-cost well tolerated drugs that can be used as cancer therapy. There is increasing evidence that drugs commonly used for benign indications, can protect against cancer incidence and mortality. Prominent examples include aspirin, metformin and sodium valproate which are currently being investigated in cancer clinical trials. Few studies have systematically screened drug libraries for anti-cancer activity in the context of gynaecological cancer. Performing such drug screens existing drugs either in isolation and or combination may identify new drugs against ovarian and endometrial cancer.

**Aims:** To screen a panel of ovarian and endometrial cell lines representative of premalignant and malignant states, with a library of 100 off patent drugs using proliferation, apoptosis assays and functional assays to identify agents which have potent anti-neoplastic properties.

**Methods:** A panel of 100 off patent drugs from the British National Formulary (BNF) will be utilised at their reported peak serum concentration to screen a panel of ovarian and endometrial cell lines encompassing both premalignant and malignant states. Post 24-72hrs incubation, cells will be assessed, first microscopically to determine any morphological changes etc, and then by Alamar Blue/MTT/BrdU assays to determine viability. Any agents that demonstrate activity will be studied...
further using a range of techniques including dose titrations to determine LC50s, apoptosis/cell death assays, morphological staining of cells etc. These will include cellular, biochemical, and molecular methods. Mode of actions will further be studied using information from the literature as a starting point.

The student will be based in Chris Tselepis’s laboratory, similar work is already underway and assays have been optimised for a variety of gastrointestinal cancer cell lines. There is also significant expertise with drug screens using these assays in haematological lineages within the University. (Farhat Khanim, School of Biosciences)

**How are you planning to ensure adequate supervision?**

The student will be fully supported through scheduled weekly meetings with the primary supervisor to discuss experiments and any concerns the student may have. The student will work closely with the supervisors and have regular access to other members of the lab. Day to day the student will work alongside an experienced postdoctoral researcher and technician who will be working on a closely related project.

**The student role.**

During the project, the student will spend most of their time engaged in laboratory-based research. Under supervision and following a period of training, they will design, conduct and interpret the results of experiments. They will also have time to read around the subject area, to understand the background to the project and to keep up to date with recent developments. The student will take an active role in weekly lab meetings/journal clubs in which they will have a chance to discuss their own data and critique the work of others.
Histone deacetylase inhibitors (HDACi) are a class of anti-cancer agents that have been approved for use in patients with a number of forms of leukaemia, but their mechanism of action remains unclear.

These reagents have been known to induce genome-wide changes in histone modification (notably histone hyper-acetylation) for many years, and their anti-cancer activity was assumed to result from the impact this would have on deregulating gene expression. However, our recent work (Halsall et al., 2012) suggests that this is not the case – only a subset of genes are affected, and those that do show changes in expression do not have changes in their histone acetylation levels.

This suggests we should explore other avenues that could explain these drugs anti-cancer activity. A recent study (Namdar et al., 2010) suggests that the genome-wide histone hyperacetylation may result in increased DNA damage which transformed cells cannot repair. We will explore this hypothesis by treating tissue culture cells with a variety of clinically relevant HDACi and assessing whether this induces DNA damage. If time permits we will compare this response in transformed and non-transformed cell lines.

This project will use a range of cell biology and molecular biology approaches, notably tissue culture, western blotting and immuno-fluorescent microscopy. If the data supports the hypothesis we will follow on with assays that assess the extent of ds-DNA breaks in cells (‘Comet assays’).

In addition the student will assist in collaborative experiments with the University of Sheffield in which xenograft models of Myeloma will be used to determine the activity of niclosamide and valproate against myeloma cells in vivo as well as their impact on M-protein levels and bone disease.

References

Marks PA (2010) Histone deacetylase inhibitors: a chemical genetics approach to
understanding cellular functions. *Biochim Biophys Acta*. 1799: 717-25 (Review)


**How are you planning to ensure adequate supervision?**

Nightingale is still research active, and will supervise directly. When not available his PhD student, or Bryan Turners post-docs will ensure day-to day lab supervision.

**The student role.**

This project will train students in a range of cell biology and molecular biology approaches, notably tissue culture, western blotting and immuno-fluorescent microscopy. If the data supports the hypothesis we will follow on with assays that assess the extent of ds-DNA breaks in cells (‘Comet assays’).

Occasional late nights and/or weekend working may be necessary to maintain tissue culture cells, but this is infrequent as lab members often cooperate.
Lead Supervisor: Dr Zania Stamataki

Co Supervisor: Professor David Adams

Project Title: T cell entosis in the liver: what happens during inflammation?

Department: Immunity and Infection

Contact Email: z.stamataki@bham.ac.uk

Telephone: None

Is the project cancer related? No

Discipline: Cancer Sciences  Immunology
Pathology  Anatomy
Metabolic Medicine  Endocrinology
Haematology  Liver & GI Medicine
Infection  Evolutionary biology in Clinical medicine

Project Outline

Entosis is the process whereby one cell invades another, and can remain internalised in its own vesicle in the host cell’s cytoplasm for long periods of time. The internalised cell may die or it may be released unharmed, and little is known regarding the molecules guiding these decisions. We recently discovered that T cells internalise into hepatocytes in the liver via entosis. The significance of this internalisation remains to be determined in health and during inflammation. The successful candidate will learn how to culture human hepatocyte and lymphocyte cell lines, label them with fluorescent markers and measure internalised cells by flow cytometry and confocal microscopy. Lymphocyte entosis will be performed in the presence of a series of proinflammatory cytokines, to help identify the conditions of lymphocyte entry into hepatocytes. The techniques used have already been established in our lab.

How are you planning to ensure adequate supervision?

The successful candidate will work directly with the primary supervisor (Dr Zania Stamataki) who is an early career research scientist that spends a lot of her time in the laboratory. The student will be trained directly by Zania and will liaise with her on a daily basis. The project will take part in the liver labs, a vibrant research environment where the student will have the opportunity to interact with postgraduate students (MRes, MD and PhD), postdoctoral scientists and clinical and non-clinical researchers. Weekly lab meetings will provide the opportunity for the second supervisor (Prof. David Adams) and other members of the lab to have input in the project, and will be also a valuable forum for the student to hear about the progress of other projects in our lab.
The student role.

The successful candidate will join a highly productive research lab with expertise in basic/translational and clinical research. Key research interests in the Liver Labs involve ongoing projects in immunology, hepatology and virology so there is plenty of opportunity for the student to sample multiple research areas. During their time in the lab, the student will be trained in laboratory techniques relevant to the project and learn how to perform experiments in a quality-controlled manner. Beyond research excellence, the intellectual contribution of the student to this project will be strongly encouraged. The student will learn to evaluate research publications and interpret results, analyse experiments and form hypotheses with the aim to foster the ability to place their research into “the bigger picture” in the field. On a daily basis, the student will plan and perform experiments, analyse results and discuss their findings in brainstorming sessions with other scientists. As the project progresses, data will be put together for presentation in scientific conferences and as part of scientific publications.
**Lead Supervisor:** Dr Zania Stamataki

**Co Supervisor**
Professor David Adams

**Project Title:** How does the liver change a T cell?

**Department:** Immunity and Infection

**Contact Email:** z.stamataki@bham.ac.uk

**Telephone:**

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Is the project cancer related? **No**

**Discipline:**
- Cancer Sciences
- Pathology
- Metabolic Medicine
- Haematology
- Infection
- Immunology
- Anatomy
- Endocrinology
- Liver & GI Medicine
- Evolutionary biology in Clinical medicine

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**Project Outline**

Modulation of T cell function is a hot topic in liver immunology. Early studies have identified the liver as a tolerising organ, as local antigen recognition occurs in the context of immunosuppressive cytokines and inhibitory cell surface ligands. These findings are difficult to reconcile with the increased expression of activation markers found in the liver compared to blood T cells. We have recently identified that interactions of CD4+ T cells with hepatocytes can alter the expression of T cell activation markers. The successful candidate will isolate human T cells from peripheral blood and co-culture them with hepatocytes in the presence or absence of proinflammatory stimuli. Alterations in T cell phenotype following co-culture will be measured using flow cytometry and confocal microscopy. The techniques used have already been established in our lab.

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<td>Co Supervisor</td>
<td>Professor David Adams</td>
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<tr>
<td>Project Title:</td>
<td>The divergent fate of B cells, T cells and NK cells in the liver.</td>
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<td>Immunity and Infection</td>
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**Project Outline**

Lymphocyte subsets reach the liver through the blood, but little is known regarding their activities and interactions with local cells in the organ. We recently discovered that hepatocytes have specialised interactions with B cells, T cells and NK cells in vitro. Live B cells and NK cells that may enter hepatocytes are transported to vacuoles that quickly become acidified, leading to lymphocyte deletion. Live T cells, however, end up predominantly in non-acidifying vacuoles and remain alive inside hepatocytes. It is intriguing that dead T cells are also efficiently deleted by hepatocytes. This project aims to dissect the mechanism behind the different fates of live lymphocyte subsets in the liver using fluorescence microscopy. The successful candidate will compare B cells, T cells and NK cells for their ability to enter hepatocytes using a series of inhibitors for adhesion molecules and endocytosis processes. The techniques used have already been established in our lab.

**How are you planning to ensure adequate supervision?**

The successful candidate will work directly with the primary supervisor (Dr Zania Stamataki) who is an early career research scientist that spends a lot of her time in the laboratory. The student will be trained directly by Zania and will liaise with her on a daily basis. The project will take part in the liver labs, a vibrant research environment where the student will have the opportunity to interact with postgraduate students (MRes, MD and PhD), postdoctoral scientists and clinical and non-clinical researchers. Weekly lab meetings will provide the opportunity for the second supervisor (Prof. David Adams) and other members of the lab to have input in the project, and will be also a valuable forum for the student to hear about the progress of other projects in our lab.
The student role.

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### Lead Supervisor:
Dr Paloma Garcia

### Co Supervisor:

### Project Title:
To study whether B-Myb has a role in DNA repair in proliferating somatic cells or ESCs.

### Department:
Immunity and Infection

### Contact Email:
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### Telephone:

### Is the project cancer related?
Yes

### Discipline:

<table>
<thead>
<tr>
<th>Cancer Sciences</th>
<th>Immunology</th>
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<td>Pathology</td>
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<td>Metabolic Medicine</td>
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<td>Haematology</td>
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<td>Infection</td>
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<td>Evolutionary biology in Clinical medicine</td>
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### Project Outline
A faulty duplication of the genome may induce lesions on the DNA that lead to mutations, genome instability or cell death when not properly repaired. We previously detected an increase in the number and intensity of H2AX· foci in embryonic stem cells (ESCs) following knock-out of B-Myb in the absence of DNA damage, and was not associated with an increase in apoptosis. Two possible mechanisms explain these effects: (i) Reduced levels of B-Myb produce collision of DNA replication forks, or (ii) B-Myb forms part of the cellular machinery responsible for chromosomal DNA repair. Our preliminary results have shown that low levels of B-Myb did not lead to accumulation of single-strand DNA breaks. Thus, we would like to investigate the role of B-Myb in DNA-repair mechanisms in somatic cells (MEFs) and ESCs in which B-Myb levels are lowered using different approaches.

### How are you planning to ensure adequate supervision?

The student role.
<table>
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<tr>
<th>Lead Supervisor:</th>
<th>Sally Jeffries</th>
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<tr>
<td>Co Supervisor</td>
<td>To be confirmed</td>
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<tr>
<td>Project Title:</td>
<td>Comparison and evaluation of genetic testing techniques for the detection of acquired genetic or genomic abnormalities in myelodysplastic syndrome.</td>
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<tr>
<td>Department:</td>
<td>West Midlands Regional Genetics Lab, Birmingham Women’s Hospital</td>
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<td>Contact Email:</td>
<td>0121 627 2710 ext 2712</td>
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**Project Outline**

Cytogenetic analysis of patients is routinely performed on patients with, or with suspected, myelodysplastic syndrome (MDS) as the chromosome abnormalities identified provide information regarding disease characterisation, risk stratification, treatment strategies and disease monitoring. G-banded metaphase chromosome analysis identifies cytogenetically visible (>10Mb) chromosome rearrangements resulting in aberrant gene activation and upregulation, deletions and copy number changes associated with the oncogenic mechanisms in MDS. However, G-band chromosome analysis is time consuming with ultimately 40~50% of MDS samples having a normal karyotype.

The project will involve comparing and evaluating the clinical utility of two new technologies, targeted next generation sequencing (NGS) and microarray analysis using molecular inversion probes (MIP array), either as stand alone, or complementary to conventional cytogenetics (G-band chromosome analysis and fluorescent in-situ hybridisation) in MDS. MIP arrays will allow detection of targeted gene mutations, copy number changes and copy-number neutral loss of heterozygosity (also called acquired uniparental isodisomy). Targeted NGS will allow mutations to be detected in targeted genes. Both these technologies detect recurrent abnormalities not detectable by conventional cytogenetics.

**How are you planning to ensure adequate supervision?**

The student will have named supervisors for both the technical and analytical work. The student will have regular project meetings with the lead or co-supervisor to review progress and discuss weekly plans.

**The student role.**

The student will be expected to perform the tests and analyse the results from both the NGS and MIP arrays. G-band chromosome analysis will be part of the routine diagnostic work up for each patient and therefore, although these results will be available, the student will not be expected to perform or analyse these tests.

The student will also be expected to perform and analyse some of the validation tests required. Validation tests are likely to include fluorescent in-situ hybridisation, short tandem repeat (STR) analysis and gene sequencing.
Project Title: Investigation of recurrent mutations of the cohesion complex in myeloid neoplasias

Department: Haematology/ West Midlands Regional Genetics Laboratory

Contact Email: susanna.akiki@bwhct.nhs.uk
Telephone: 0121 626 4521

Is the project cancer related? Yes

Discipline: Cancer Sciences, Pathology, Metabolic Medicine, Haematology, Infection, Immunology, Anatomy, Endocrinology, Liver & GI Medicine, Evolutionary biology in Clinical medicine

Project Outline

Acquired mutations and deregulated expression of genes that regulate growth and differentiation in haematopoietic progenitor cells underlies the pathogenesis of leukaemia. A comprehensive understanding of the complexity of leukaemia genetics is therefore essential for understanding the mechanisms of disease, accurate prediction of outcome and the development of novel, rationally targeted therapies. Cohesion is a multimeric protein composed of four subunits which is involved in cohesion of sister chromatids in mitosis, post replicative DNA repair and regulation of gene expression. Recurrent mutations have recently been reported in coexistence with other common mutations in myeloid neoplasias and impaired function is implicated in leukemogenesis, possibly through deregulated expression inducing DNA hypermutability. (Welch; Cell 150, 264-278; 2012; Kon; Abstract No: 782; ASH 2012)

The aim is to investigate cohesion mutations in a series of myeloid neoplasia’s using next generation sequencing together with SNP arrays to interrogate copy number changes. Where possible the analysis would be included with existing genomic profiling data to more fully characterise the spectrum of mutations associated with myeloid neoplasia’s which is not yet complete. Reports of cohesion mutated cases, to date are from cases with an apparently normal karyotype therefore screening for this novel class of genetic targets in myeloid malignancies represents a potentially important prognostic marker in myeloid malignancies in addition to highlighting a possible role of compromised cohesion function in myeloid...
leukaemogenesis.

**How are you planning to ensure adequate supervision?**

Experimental design, process and data analysis would be carried out under the supervision of Dr Akiki in the West Midlands Regional Genetics laboratory which as as a large clinical laboratory already has existing expertise and equipment to enable completion of the project outlined here. Clinical supervision, selection of cases and data interpretation would be supervised by Dr Raghavan in haematology.

**The student role.**

To carry out retrospective profiling on a series of primary myeloid malignancies at diagnosis from the West Midlands Regional Genetics Lab to correlate mutation spectrum with clinical outcome for this new class of genetics targets in myeloid malignancies. This would require

- training in genetic technologies that would allow the student to design and carry out an appropriate screening strategy to interrogate mutations and copy number changes in genes encoding components of the cohesion complex.
- bioinformatics support to analyse and interpret the data generated for correlation with clinical outcome.
- The student to keep up to date with emerging literature in this newly developing area and feedback developments into the project
- To prepare the data in a form that would be suitable for presentation at an external meeting
Lead Supervisor: Phil Newsome

Co Supervisor: Jon Frampton

Project Title: Mesenchymal stem cells as anti-inflammatory therapeutics in liver disease

Department: Centre for Liver Research

Contact Email: P.N.Newsome@bham.ac.uk
Telephone: 0121-415-8700

Is the project cancer related? No

Discipline: Cancer Sciences  Immunology
Pathology  Anatomy
Metabolic Medicine  Endocrinology
Haematology  Liver & GI Medicine
Infection  Evolutionary biology in Clinical medicine

Project Outline

My group has a major interest in the ability of mesenchymal stromal cells (MSC) to suppress immune-mediated liver injury. My group have demonstrated the ability of MSC to reduce damage in simple forms of liver injury.

My group have established the isolation of a purified population of mouse MSC which have greater immunosuppressive functionality.

This project will examine the action of MSC in a model of alcohol induced liver damage. The student will help study the model of alcohol induced liver damage in the NRF-2 ko mouse model.

Studies will include:
1: Test efficacy of MSC in model of alcoholic hepatitis.
2: Study the molecular mechanism by which MSC exert their effect
3: Define the location of action of MSC by carrying out tracking studies.

How are you planning to ensure adequate supervision?

Weekly meeting with the main supervisor.
Will be working alongside a PhD student and 2 post-docs.

The student role.

Performing murine MSC isolation alongside the PhD student.
Analysing the outcome of murine experiments after MSC infusion.
Analysing murine samples to determine severity of liver damage.
Carrying out tracking studies with labelled MSC.
Project Outline

Seminoma is the most common form of testicular cancer and responds very well to surgery and chemotherapy. Our group is interested in studying how the immune response may play a role in the helping to eradicate seminoma and it is of interest that a T cell infiltrate is found in every case of seminoma. Indeed, this is a useful pointer to making the diagnosis.

In the last 2 years we have collected over 50 serial serum samples from patients who are undergoing treatment for seminoma. These have been stored within the laboratory and have not yet been studied in a research project. This proposal has two aims:

- The first is to use the serum samples to see if there is a humoral (antibody) immune response to testicular protein. This will be accomplished by immunohistochemical analyses of testicular tissue using expertise that is freely available in the Pathology laboratory. Serum samples, both controls and patient-derived, will be incubated with testicular tissue from both normal and tumour-associated cases and the potential staining of tissue will be viewed using antibody staining. This will be correlated with clinical outcome.

- The second phase of the project will involve detailed immunohistochemical analysis of the nature of the T cell infiltrate in seminoma. We have been able to isolate live T cells from seminoma and show that these have unusual features that are typical of ‘exhausted’ T cells. This is likely to reflect immune evasion by the testicular tissue and tumour. Here we seek to extend these findings to study a large number of paraffin-sections of seminoma tissue. We will seek to investigate if the number or architecture of the T cell infiltrate correlates with tumour morphology or clinical outcome.
**The student role.**

- Work to develop immunohistochemical analysis of testicular tissue
- Utilise serum samples collected from patients with testicular cancer
- Develop expertise in immunohistochemistry, and potentially confocal microscopy, to study the T cell infiltrate in seminoma
The liver consists of many cell types but is composed mainly of epithelial cells, i.e., hepatocytes and biliary epithelial cells, or cholangiocytes. These biliary cells are cuboidal epithelial cells and account for around 5% of liver mass. The biliary tree consists of different size ducts within the liver, these being large septal ducts, medium-sized interlobular ducts and small ducts or ductules, which are located within portal tracts consisting of a bile duct, hepatic artery and portal vein with blood flowing in the opposite direction to bile. Cholangiocytes are also controlled by adrenergic and cholinergic nerves. The main function of healthy cholangiocytes is to modify bile before it reaches the intestine but they also have important immune functions and are involved in tissue homeostasis. Both hepatocytes and cholangiocytes are derived from a common progenitor cell during embryogenesis and there are thought to be a compartment of progenitor cells which persist in the adult liver.

During disease the bile ducts can become injured and there are diseases which specifically target bile ducts; the cholangiopathies. There are many causes of cholangiopathy such as immune-mediated disease, infection or drug-induced disease. A common feature of cholangiopathies is inflammation, which may be resolved. If there is persistence, progression and chronic inflammation then cholestasis, biliary proliferation and ductopenia may develop. This may ultimately lead to fibrosis and malignant transformation into cholangiocarcinoma. During disease, cholangiocytes (mainly in ductules) can become activated and more reactive in a process termed ductular reaction. These reactive cells are more likely to secrete cytokines, chemokines and growth factors which exacerbate portal inflammation by immune cells. They can also secrete factors to neighbouring endothelial cells to promote angiogenesis as well as activating myofibroblasts which are involved in fibrogenesis. These ductular reactive cells are thought to originate from the progenitor population within the liver and it is also thought that these cells may be more plastic in their phenotype and function than mature cholangiocytes.

Hypothesis: Mature cholangiocytes are fully-differentiated, non-reactive cells that differ from ductular reactive cells and cholangiocarcinoma cells in their phenotype and function as ductular reactive cells and malignant cells may be more like bipotent...
progenitor cells.

Aim: to assess cholangiocyte phenotype and function in mature ducts, ductular reactive cells and cholangiocarcinoma cells.

### How will you ensure adequate supervision?

Being a post-doctoral research fellow I spend the majority of my time in the laboratory thus I will be able to teach and assist with any technical issues that may arise. The student will also have the opportunity to participate with the rest of the Afford group in fortnightly lab meetings as well as having one to one meetings with myself and Dr Afford, when required, to discuss experimental design, data analysis and the direction of the project.

### The student role.

The student will be based in the Centre for Liver Research where they will perform immunohistochemistry on a variety of end stage non-malignant liver tissue sections to phenotype mature cholangiocytes and ductular reactive cells, and also cholangiocarcinoma tissue. Primary cholangiocytes will be isolated from normal donor tissue or end stage liver disease tissue by positive immunomagnetic selection and cultured. The primary cells along with non-malignant and cholangiocarcinoma cell lines will be phenotyped by immunocytochemistry and flow cytometry using epithelial, endothelial, mesenchymal and stem cell markers to ensure culture purity and assess cell lineage. Cell polarity will be assessed by immunofluorescent staining and transwell experiments. Growth factor-dependent cell signalling will be examined by western blotting. The secretome will be assessed by multiplex and metabolic analysis will be performed by mass spectroscopy.
**Lead Supervisor:** Kai-Michael Toellner

**Co Supervisor** Yang Zhang

**Project Title:** The role of Immunoglobulin class switching for immunological memory.

**Department:** Immunity and Infection

**Contact Email:** Tel: 58687, K.M.Toellner@bham.ac.uk, Y.Zhang.10@bham.ac.uk

**Is the project cancer related?** Yes or No

**Discipline:**
- Cancer Sciences
- Pathology
- Metabolic Medicine
- Haematology
- Infection
- Immunology
- Anatomy
- Endocrinology
- Liver & GI Medicine
- Evolutionary biology in Clinical medicine

**Project Outline**

Immunoglobulin class switch recombination (CSR) is induced during initial B cell activation, and again while B cells differentiate in germinal centres. CSR not only changes the type of antibody a B cell can secrete, but also the B cell receptor. There are several pathways how a B cell can differentiate to become a memory cell (Taylor, 2012), and these may or may not involve CSR (Marshall, 2011). We plan to test how CSR determines fate of memory B cells in a new mouse model: mice that are double transgenic for a CSR reporter gene (Casola, 2006) and a gene that makes these cells susceptible to diphtheria toxin. In these mice B cells that have undergone activation and CSR can be killed specifically at defined times during the immune response. Analysis of immune responses in these mice will give us information on the efficiency of switched and non-switched memory B cells to differentiate into memory B cells and participate in recall responses.

**Key References:**

**How are you planning to ensure adequate supervision?**
The student will be supervised in meetings, discussions, and review of results on a daily basis by KMT and Yang Zhang, a postdoctoral research fellow who has done preliminary experiments on this project and is familiar with all techniques involved. All necessary techniques are established and will be learned by training through members of the research groups of KMT. Weekly group meetings will give opportunity to train presentation and discussion skills.

**The student role.**
The student will conduct and analyse all experiments, supervised by postdoctoral researchers and KMT. Some animal experiments will form part of the project. A home office license is not prerequisite for the project, as procedures on animals can be performed by other members of the research group.
**Lead Supervisor:** Kai-Michael Toellner

**Co Supervisor** Yang Zhang, Roy Bicknell

**Project Title:** The role of Immunoglobulin class switching for immunological memory.

**Department:** Immunity and Infection

**Contact Email:** Tel: 58687, K.M.Toellner@bham.ac.uk, Y.Zhang.10@bham.ac.uk

**Is the project cancer related?** Yes

**Discipline:**
- Cancer Sciences
- Pathology
- Metabolic Medicine
- Haematology
- Infection
- Immunology
- Anatomy
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**Project Outline**

Vaccination against cancer have been developed over recent years. While preventative vaccines have been introduced for some cancers, therapeutic vaccination is still in its infancy. We have developed a vaccine that targets antigens widely expressed in vessels of tumour tissues: the lower shear stress in tumour vessels compared to vessels of normal tissues leads to expression of the tumour endothelial cell antigens Robo4 and Clec14a specifically in tumour tissues (Heath and Bicknell, 2009; Mura et al., 2012). While most current cancer specific vaccines have been designed to induce cytotoxic T cell responses, we decided to develop a protocol that induces a strong and reliable antibody response. This was achieved by linking the tumour specific auto-antigen to specific components that lead to efficient tumour-specific antibody production.

We have shown in preliminary experiments that vaccination with our vaccine induces a rapid anti-tumour vessel antibody response. This led to reduced tumour growth in a rapidly growing Lewis lung carcinoma model implanted into a subcutaneous sponge, even when the vaccine was given at the time of tumour implantation. Mice deficient in B cells have tumour growth identical to non-vaccinated mice, showing that protection is dependent on antibody (data submitted for publication).

We now want to translate this vaccine protocol to components used in human vaccination. The project will involve producing new vaccines by genetic engineering, and initially immunising mice with them to test whether they show similar efficiency to our original constructs.


### How are you planning to ensure adequate supervision?

The student will be supervised in meetings, discussions, and review of results on a daily basis by KMT and Yang Zhang, a postdoctoral research fellow who has done preliminary experiments on this project and is familiar with all techniques involved. All necessary techniques are established and will be learned by training through members of the research groups of KMT and Roy Bicknell. Weekly group meetings will give opportunity to train presentation and discussion skills.

### The student role.

The student will conduct and analyse all experiments, supervised by postdoctoral researchers and KMT. Some animal experiments will form part of the project. A home office license is not prerequisite for the project, as procedures on animals can be performed by other members of the research group.
Lead Supervisor: Miss Saaeha Rauz
Co Supervisors: Professor Ann Logan
Dr Graham Wallace
Project Title: The role of decorin in modulating ocular surface fibrosis.
Department: Centre for Translational Inflammation Research (QEHB)
Section of Neurotrauma and Neurodegeneration (IBR West)
Contact Email: s.rauz@bham.ac.uk; a.logan@bham.ac.uk; g.r.wallace@bham.ac.uk
Telephone: 0121-371-3254/3255; 0121-414-8855
Is the project cancer related? Yes
Discipline: Cancer Sciences Immunology
Pathology Anatomy
Metabolic Medicine Endocrinology
Haematology Liver & GI Medicine
Infection Evolutionary biology in
Clinical medicine

Project Outline

Background: The integrity of the highly specialised ocular surface (OcS) mucosal barrier is crucial for optical clarity, eyesight, ocular protection and health. It consists of three distinct areas: the conjunctiva, corneoscleral limbus and cornea that are interrelated with adnexal structures (eyelids, lashes, lacrimal system) to form the integrated lacrimal functional unit. Triggers such as infections and immune-mediated challenges result in OcS breakdown and ultimately OcS scarring - a leading cause of worldwide blindness with an estimated 16 million people known to be visually impaired (WHO, 2004). The OcS mucosal stromal microenvironment is known to comprise of tissue-specific stromal cells (fibroblasts (conjunctiva), keratocytes (cornea), resident macrophages), and a highly specialised extracellular matrix (ECM), that together provide the prelude to site-specific responses. During the initiation of inflammation, fibroblasts are activated by various pro-inflammatory signals produced from activated leukocytes. Activated fibroblasts in turn, produce numerous cytokines and chemokines, such as interferon-beta (IFNβ), stromal derived factor-1alpha (SDF-1α / CXCL12) and transforming growth factor-beta (TGF-β1) which alter the capacity of the stromal microenvironment to mediate survival and retention of specific immune cells. The tissue-specific stromal microenvironment orchestrates the withdrawal of survival signals and normalisation of chemokine gradients that enable eradication of infiltrating cells, but not without damage to optical clarity by fibrosis. TGF-β1 is known to be one of the main inducers of fibroblast differentiation into myofibroblasts which persist after the initial wound healing response, modulating the ECM with subsequent potentially visually significant scar formation. Strategies to mitigate the acute phase of OcS fibrosis remains a major unmet global health need. One putative candidate is Decorin, a naturally occurring extracellular small leucine rich proteoglycan that interacts with ECM molecules, growth factors and their receptors, including the TGF-βs. Decorin sequesters TGF-βs into the ECM thereby prohibiting interaction with TGF-β cell surface receptors reducing deposition of chondroitin sulphate proteoglycans, fibronectin and laminin. Our group has shown that GMP grade Decorin may have a critical role in regulating CNS and trabecular meshwork fibrosis with phase I clinical trials pending for mediating post-traumatic fibrosis during neurosurgery and glaucoma filtration surgery, respectively. This project will examine the novel role of Decorin on in vitro models of OcS fibrosis to provide preliminary evidence that Decorin has the potential to impact on global blindness secondary to OcS disease.
Plan of investigation: Primary cultures of corneal and conjunctival fibroblasts will be derived from redundant human corneal transplant material and biopsies of healthy conjunctiva (ethics in place) using protocols established by our group. Using a combination of (i) proliferation, (ii) migration (iii) myofibroblast differentiation assays, fibroblasts will be challenged with TLR3, 4, TNF-α in the presence or absence of TGF-β1 ± Decorin. Dose-response experiments will define optimal Decorin concentrations necessary to inhibit and reverse myofibroblast differentiation accompanied by phase contrast microscopy to quantify changes in cellular density (reflecting cell proliferation and migration). Alpha-MSH and Hoechst positive cells will be define the presence of myofibroblasts.

To assess matrix contraction (a measure of myofibroblast differentiation and ECM production), free-floating, relaxed collagen gel lattice models will be used by populating three-dimensional collagen constructs with fibroblasts and feeding with TGF-β1 ± Decorin. The reduction in lattice area at days 1, 3, and 7 will be digitally photographed for measurement. All experiments will be repeated utilising conjunctival fibroblasts derived from conjunctival biopsies taken from patients with pro-fibrotic ocular disease such as ocular mucous membrane pemphigoid, an immune mediated progressive conjunctival scarring disorder that leads to a blinding keratopathy.

How are you planning to ensure adequate supervision?

The Academic Unit of Ophthalmology (Miss Rauz, Dr Wallace) research laboratories are located in the Centre for Translational Inflammation Research in the University Laboratories at the QEHB whereas Professor Logan is based at the IBR-West in the neighbouring medical school. Clinical samples will be obtained from surgical procedures carried out at the Birmingham and Midland Eye Centre (the second largest eye hospital in the UK) where existing ethics encompasses all clinical aspects of this proposal. All cell culturing and experimental techniques are established in both groups. Day to day laboratory support will be provided by post-doctoral researchers and senior students. Weekly meetings will be held with all three supervisors to ensure adequate student/project progression and for troubleshooting. In addition the student will attend both Ophthalmology and Neurobiology research seminars. Given the combination of cross-discipline clinical and non-clinical supervision, the student will be exposed to a number of transferable research skills including training in ethics, clinical phenotyping with outcome measures, data analyses, scientific writing and presentation skills.

The student role.

The student is expected to liaise with surgeons for tissue samples, anonymise, track tissues from the operating room to the labs, and carry out all experiments and data analyses. In the first instance this will be with guidance but toward the end of the project there will be more autonomy. They will present data at the weekly lab meetings and to the wider group. They will also be expected to conduct a detailed literature review of the background to the research project, assimilate published techniques and how these might apply to the project and discuss with the supervisors. Writing skills will be developed for thesis preparation and manuscripts for scientific submission. The student should follow SOPs and the principles of the health and safety at work, good laboratory practice and good clinical practice must be adhered to at all times. Team working and interacting with peers and senior colleagues will be required.