UNIVERSITY OF
BIRMINGHAM

College of Medical and Dental Sciences

Intercalated BMedSc Clinical Sciences

Research projects 2012/13

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** or please contact the course administrator, Miss Yvonne Palmer in the Division of Cancer Studies: 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.

Primary Supervisor:	Dr. F. Berditchevski, Dr. E. Odintsova
Project Title:	**Regulation of responses of breast cancer cells to Herceptin- and Lapatinib-based therapies.**
Department:	School of Cancer Sciences
Contact:	Email: f.berditchevski@bham.ac.uk
Discipline:	Cancer Sciences [ ] V Pathology [ ] Metabolic Medicine [ ] Haematology [ ] Infection [ ] Immunology [ ] Anatomy [ ] Endocrinology [ ] Liver & GI Medicine [ ] Evolutionary basis of clinical medicine [ ]

Project Outline

Transmembrane receptors of ErbB1/Her1 and ErbB2/Her2 are overexpressed in 15-25% of breast cancers and this correlates with poor prognosis. A number of drugs have been developed which target these proteins, including Herceptin/Trastuzumab, Cetuximab, Lapatinib. Whilst 40-60% patients have a short term benefit from using these drugs, many of them will eventually develop resistance to the treatment. Furthermore, a significant proportion of patients do not respond to these drugs. Therefore, more work is required to understand the molecular mechanisms underlying drug-resistance in breast cancer.

Research in our laboratory has shown that targeting protein complexes which include tetraspanins can affect responses of breast cancer cells to Herceptin- and Lapatinib-based therapies. Tetraspanins are transmembrane proteins which function as organisers of the specialised microdomains/molecular aggregates at plasma membrane. We have recently identified peptides which may specifically affect tetraspanin functions and, consequently, they may influence how breast cancer cells respond to the treatments.

The specific aims of the project are:
a) to examine responses of breast cancer cell lines to the peptides which target tetraspanins in combination with anti-ErbB1/2 drugs (e.g. proliferation and migration assays);
c) to investigate the activity of ErbB receptors in treated cells.

How are you planning to ensure adequate supervision?

We supervise intercalating students on a daily basis in the lab, provide training in necessary techniques and ensure that students have a firm theoretical grasp of the project. .
The student role.
After training is provided, the student is expected to carry out experiments independently, to analyse the results and to discuss the outcome of the experiments and future applications of the acquired knowledge. The project is a part of the ongoing research in the lab, all participants will be included in the publication of the results when the time comes.

Primary Supervisor:	Victoria Heath
Project Title:	Development of methods to inhibit tumour angiogenesis
Department:	School of Immunity and Infection
Contact:	Email: Tel: 0121 415 8818
Discipline:	Cancer Sciences ■ Pathology ☐ Metabolic Medicine ☐ Haematology ☐ Infection ☐ Immunology ☐ Anatomy ☐ Endocrinology ☐ Liver & GI Medicine ☐ Evolutionary basis of clinical medicine ☐

Project Outline

The growth and metastasis of solid tumours depends on their developing their own blood supply. Angiogenesis, the development of new vessels from the pre-existing vasculature, is mediated by endothelial cells, the cells that line blood vessels. There is considerable effort being put into the development anti-angiogenic strategies to treat cancer, and the first such licensed agent Avastin (anti-vascular endothelial growth factor) entered the clinic six years ago (1). Our laboratory aims to identify novel genes that play a role in angiogenesis, particularly in cancer, using new sequencing technologies and bioinformatic techniques. These candidate genes are then investigated for their role in endothelial motility and tube formation using a combination of siRNA-mediated knockdown of genes of interest and determining how this affects endothelial cells and a range of in vitro assays. A number of different methods and models have been developed to understand how genes of interest play a role in angiogenesis in vivo and the aim of this project is to develop some of these for testing the roles of novel genes in angiogenesis.

Two models will be explored. The first involves the use of retrovirus producing cells to transduce the mouse vessel endothelial cells with short-hairpin RNA to knockdown genes of interest. These producer cells are then co-implanted with human tumour cells into immunocompromosed mice to allow the assessment of the role of the gene in the host endothelial cells without affecting the human tumour cells (which can not be infected with this virus) (2). The second model involves subcutaneous implantation of a solubilised basement membrane preparation into which vessels grow over the course of a week. In order to knockdown gene expression small inhibitory (si)RNA will be added into the matrigel. This project will give the student a range of experience with molecular biology, cell culture and in vivo tumour and angiogenesis models, using state of the art techniques to manipulate gene expression.

1) Heath and Bicknell. 2009. Anti-cancer strategies involving the vasculature. Nat Rev Clin Oncol. 6:395-404
2) Mavria et al., 2006. ERK-MAPK signaling opposes Rho-kinase to promote endothelial cell survival and sprouting during angiogenesis. Cancer Cell 9:33-44.

How are you planning to ensure adequate supervision?

I will be supervising the student myself in the lab for the majority of the project and on occasions that I am not available others in the lab will be able to assist. .
The student role.
The student will undertake the experiments outlined, having been trained by the supervisor. Since this project involves in vivo work they will have to be appropriately trained and acquire a personal home office licence for working with animals.

Primary Supervisor:	Dr Jo Parish
Project Title:	Analysis of the interaction of HPV16 E2 with the Rad50 interacting protein Rint1 and E2-dependent modulation of the cellular DNA damage response.
Department:	School of Cancer Sciences
Discipline:	Cancer Sciences [x] Pathology [ ] Metabolic Medicine [ ] Haematology [ ] Infection [ ] Immunology [ ] Anatomy [ ] Endocrinology [ ] Liver & GI Medicine [ ] Evolutionary basis of clinical medicine [ ]

Project Outline

Background

Papillomaviruses (PVs) infect epithelial cells and can result in the formation of benign warts that are often persistent and hard to treat. However, infection with some PV types can also cause cellular transformation. Human PV (HPV) infection is the cause of almost 100% of cervical carcinomas and a major cause of other anogenital and oropharyngeal carcinomas. PVs have a small circular double-strand DNA genome that is maintained in host cells independently of the cellular chromosomes. Replication, maintenance and amplification of PV genomes require expression of the viral helicase E1 and the replication initiation and transcriptional control factor E2. It has recently been shown that the oncogenic HPV31 manipulates cellular DNA damage responses (DDR) to facilitate viral genome amplification in differentiating keratinocytes[1] and that unlike the replication of other double-stranded DNA viruses, HPV replication is not arrested by activation of DDR[2]. Furthermore, an increase in the DNA damage foci, double strand DNA breaks and genome instability has been reported in cervical lesions. It has been reported that expression of E1 is sufficient to cause activation of the DDR, and subsequent accumulation of γ-H2AX foci. Interestingly, co-expression of E2 with E1 enhances the formation of damage foci[3], indicating an E2-specific function in DDR activation. These studies have led to the hypothesis that E1 and E2 expression causes cellular DNA damage, halting cellular but not HPV DNA replication, facilitating amplification of viral genomes. This may facilitate integration of HPV genomes, an important step to HPV-induced carcinogenesis. However, the mechanism by which E1 and E2 manipulate the cellular DDR is unknown.

Using a yeast 2-hybrid screen to isolate bovine PV (BPV1) E2 interacting proteins, we isolated Rad50-interacting protein 1 (Rint1) as a binding partner of E2. Rint1 has been shown to associate with Rad50 via its C-terminal domain during late S and G2/M phases and over-expression of this domain of Rint1 causes defective G2/M checkpoint activation[4]. We have confirmed the interaction between the E2 proteins from diverse PV types and Rint1. Furthermore, we have evidence that expression of HPV16 E2 results in a significant destabilization of Rint1 protein. **We hypothesize that this represents a novel mechanism by which PVs interfere with the DDR, causing delayed repair of damage lesions, which would inhibit progression through the G2/M checkpoint and thus facilitate viral genome amplification. To test this hypothesis, we will further characterize the interaction between E2 and Rint1 and determine the function of this interaction in the viral life cycle.**

Plan of investigation

Aim 1: Characterisation of the interaction between E2 and Rint1

Since Rint1 and Rad50 specifically associate during late S and G2/M phases, it will be important to determine whether E2 targets Rint1 during these specific stages using synchronized cell cultures. In addition, it has been shown that E2 protein is specifically phosphorylated during S phase resulting in cell cycle-specific stabilisation and therefore it will be established if the interaction between E2 and Rint1 is regulated by E2 phosphorylation. This will be done by treating cells with specific kinase inhibitors before harvesting, or by treating cell lysates with phosphatase before immunoprecipitation.

Aim 2: Characterization of the function of E2:Rint1 interaction in DDR

Expression of E2 protein has been shown to destabilize endogenous Rint1(Parish, unpublished). Whether E2-dependent destabilisation of Rint1 is an important step in the activation of the DDR in E1 and E2 expressing cells has yet to be determined. Therefore, the function of the interaction between E2 and Rint1 on activation of the DDR will be determined by either over-expression of Rint1 protein or RNA interference-mediated depletion of Rint1 in E1 and/or E2 expressing cells. We hypothesize that a change in the steady state levels of Rint1 protein in E1 and E2 expressing cells will affect E1 and E2-induced DNA damage foci and activation of the cellular DDR. This is a hypothesis we will test.

1. Moody, C.A. & Laimins, L.A. Human papillomaviruses activate the ATM DNA damage pathway for viral genome amplification upon differentiation. *PLoS Pathog.* **5**, e1000605 (2009).
2. King, L.E. et al. Human papillomavirus E1 and E2 mediated DNA replication is not arrested by DNA damage signalling. *Virology* **406**, 95–102 (2010).
3. Sakakibara, N., Mitra, R. and McBride, A.A. The Papillomavirus E1 Helicase Activates a Cellular DNA Damage Response in Viral Replication Foci. *J Virol*. **85**, 8981–8995 (2011).
4. Xiao, J., Liu, C.C., Chen, P.L. & Lee, W.H. RINT-1, a novel Rad50-interacting protein, participates in radiation-induced G(2)/M checkpoint control. *J. Biol. Chem.* **276**, 6105–6111 (2001).

How are you planning to ensure adequate supervision?

All students under my supervision meet with me weekly to discuss progress and short and long-term plans. In addition, students in my laboratory attend weekly lab meetings in which a member of the group presents their most recent experimental data. This is then discussed in an informal and supportive manner. I also facilitate frequent journal club style meetings in which students and postdocs present a recent publication in our area of interest. This provides useful discussion amongst the group and provides an opportunity for feedback on presentation and critical analysis skills.

Students are also informally supervised within the laboratory by either an experienced postdoctoral research assistant or myself. This provides a firm support network for students in the laboratory that can be adapted to an individual's specific needs.

Primary Supervisor:	Z Nagy, R Bicknell, G Brown
Project Title:	Effect of Retinoic Acid Receptor Antagonists on Neurones and Angiogenesis
Department:	School of CEM
Contact:	Email: z.nagy@bham.ac.uk Tel: 0121 415 8135
Discipline:	**Cancer Sciences**

Project Outline

Neurones play a role in Alzheimers 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 know 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 RAR·, RAR·, and RAR·. These receptors function as ligand-dependent transcriptional regulators and form heterodimers with retinoid X (or rexinoid) receptors (RXR·, ·, and ·). 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 RAR· drives the differentiation and maturation of primitive haematopoietic cells. The function of RAR· is particular 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. **These agonists 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.

Figure 1

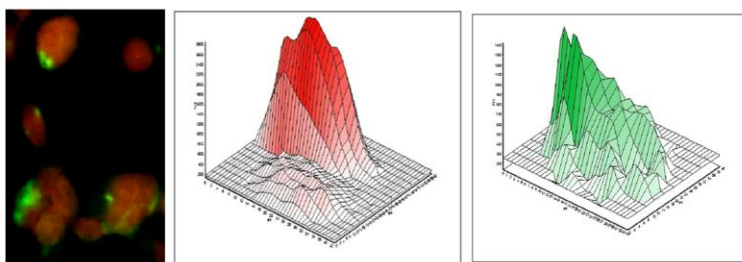
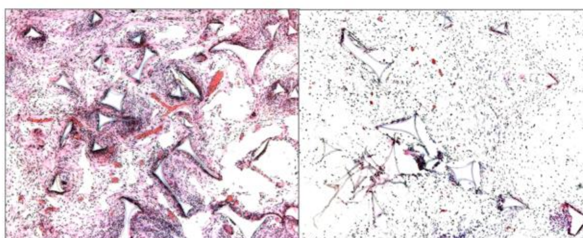


Figure 2

Figure 3



[1] Samarut E, and Rochette-Egly C. Nuclear retinoic acid receptors: Conductors of the retinoic acid symphony during development. Mol Cell Endocrinol. 2011;doi: 10.1016/j.mce.2011.03.025

[2] Lee HP, Casadesus G, Zhu X, Lee HG, Perry G, Smith MA, Gustaw-Rothenberg K, Lerner A. All-trans retinoic acid as a novel therapeutic strategy for Alzheimer's disease. Expert Rev Neurother. 2009 Nov;9(11):1615-21.

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 us and the technologies used for the completion of the project are state of the art, the possibility of producing novel findings is high.

Primary Supervisor:	Professor Chris McCabe and Dr K Boelaert
Project Title:	The role of PBF in Epithelial to Mesenchymal Transition (EMT) in breast cancer
Department:	School of Clinical and Experimental Medicine
Contact:	Email: k.boelaert@bham.ac.uk Tel: 0121 415 8712
Discipline:	Cancer Sciences ■ Pathology □ Metabolic Medicine □ Haematology □ Infection □ Immunology □ Anatomy □ Endocrinology ■ Liver & GI Medicine □ Evolutionary basis of clinical medicine □

Project Outline

Most human breast cancers evolve from normal epithelial cells through a series of increasingly abnormal stages over long periods of time. Key stages in this progression are hyperplasia, atypical hyperplasia, *in situ* carcinoma, invasive carcinoma and, finally, metastatic disease. Invasion into surrounding stroma defines the transition from *in situ* to invasive carcinoma. However, most defects responsible for the development and progression of malignant disease remain unknown.

We recently elucidated a role for the pituitary tumor transforming gene binding factor (PBF) in breast cancer, which identified this gene as a potential novel therapeutic target (1). Briefly, we showed that PBF mRNA and protein expression were induced by estradiol in ER•-positive MCF-7 cells. PBF expression was low or absent in normal breast tissue, but was highly expressed in breast cancers, where it correlated with ER• status. Critically, PBF induced invasion through Matrigel, independently of proliferative changes, and which could be abrogated both by siRNA treatment and specific mutation.

Metastasis is responsible for the vast majority of breast cancer deaths (12), and is preceded by cell invasion and the infiltration of invasive cells throughout the surrounding tissue. Our existing data demonstrate that PBF is a potent invasive gene in vitro. We now wish to determine the genetic changes which lie downstream of the induction of cell invasion by PBF. We will isolate invasive (lower layer) and non-invasive (upper layer) MCF12A breast cells from Matrigel cell invasion assays. After purification and RNA extraction, we will perform Human Epithelial to Mesenchymal Transition (EMT) focussed cDNA arrays, which contain 84 genes specifically involved in metastasis and invasion, allowing us to identify those EMT genes demonstrating altered expression in response to PBF transfection which result directly in cell invasion. Those genes showing significant changes in expression in invasive vs. non-invasive MCF12A cells will be validated through TaqMan RT-PCR and Western blotting.

Taken together, therefore, this project will address the process of Epithelial to Mesenchymal Transition in breast cancer, a critical step in tumour malignancy. Specifically, we will examine the influence of the novel proto-oncogene PBF on the regulation of genes which underpin EMT and cell invasion.

How are you planning to ensure adequate supervision?

The student will join the McCabe/Boelaert group, a vibrant mix of basic and clinical scientists within the Institute for Biomedical Research. Supportive and responsive supervision will be provided by Professor Chris McCabe and Dr Kristien Boelaert (Academic Consultant Endocrinologist), experienced post doctoral fellows (Dr Martin Read, Dr Vicki Smith), and several PhD students within the lab. In addition to daily supervision, we have fortnightly lab meetings where data and findings are discussed. The student will be encouraged to attend other fora such as journal clubs and seminars for a more generic exposure to endocrinology and cancer.

The student role.

Following an initial period of laboratory training, the student will embark upon a series of experiments, under the supervision of experienced scientists. The project will provide extensive training in a number of techniques employed in molecular endocrinology. These include cell culture, invasion assays, DNA, RNA and protein extraction, cDNA array analysis, TaqMan RT-PCR and Western blotting. Additionally there will be training in more general research methodology, statistical analysis, scientific writing and presentation skills. As the project progresses, the student will be encouraged to drive the project through their own initiative, and to explore their own ideas. We hope the student will contribute to the close working environment of the McCabe/Boelaert group, and it is anticipated that data will be obtained which are useful in the context of the project, but which are also publishable, and will result in joint publications and conference submissions.

Primary Supervisor:	Dr Agnieszka Gambus
Project Title:	Novel roles of SUMO modifiers in genome maintenance
Department:	School of Cancer Sciences
Contact:	Email: a.gambus@bham.ac.uk Tel: 0121 414 7984
Discipline:	Cancer Sciences ■ Pathology ☐ Metabolic Medicine ☐ Haematology ☐ Infection ☐ Immunology ☐ Anatomy ☐ Endocrinology ☐ Liver & GI Medicine ☐ Evolutionary basis of clinical medicine ☐

Project Outline

Proper genome maintenance is crucial for normal development and prevention of premature aging and diseases such as cancer. Cells are very often exposed to genotoxic insults and to protect their genome integrity they have developed elaborate DNA damage response machinery. A new critical element of this machinery has been described within the last few years: modification of proteins by SUMOs (small-ubiquitin like modifiers). The process of SUMOylation has been shown to be essential in response to a number of different types of DNA damage. However the protein substrates modified in each case are often still unknown.

The aim of this project is therefore to investigate further the role of SUMO family members during replication stress and DNA damage response. We will test the requirement for SUMOylation in response to the types of DNA damage in which it has not yet been implicated and characterise the defect caused. We will also aim to identify novel substrates of SUMOylation during DNA damage processing.

To conduct this research, we 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.

Bartek J, Hodny Z.
SUMO boosts the DNA damage response barrier against cancer.
Cancer Cell. 2010 Jan 19;17(1):9-11. Review.

How are you planning to ensure adequate supervision?

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. Although I have not supervised the intercalated research project before, I did supervise an honors project student at the University of Dundee with very good results – my student has been awarded a price for the best project within her year. .
The student role.
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 I will use for future grant applications and publications. The Student will be a co-author of any publication resulting from this project.

Primary Supervisor:	P-S Jayaraman
Project Title:	Hypoxia and the Regulation of tumour cell growth/migration by the PRH transcription factor
Department:	School of Immunity and Infection
Contact:	Email: p.jayaraman@bham.ac.uk
Discipline:	Cancer Sciences ☐ x Immunology ☐ Pathology ☐ Anatomy ☐ Metabolic Medicine ☐ Endocrinology ☐ Haematology ☐ Liver & GI Medicine ☐ Infection ☐ Evolutionary basis of clinical medicine ☐

Project Outline

Understanding the molecular mechanisms that control cell proliferation and cell differentiation is central to understanding tumourigenesis and work in this area has laid the foundations for targeted cancer therapies. 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 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 controls tumour growth and tumour cell migration. Our recent data has shown the PRH can antagonise the HIF-1 protein at the VEGFR-1 promoter under normoxia.
We are interested to determine whether PRH is down regulated at the mRNA or protein level under hypoxia when HIF-1 activity would be stabilized. To investigate this we will measure PRH mRNA levels in a variety of normal and tumour cell lines in normoxia and hypoxia. We will also examine the effects of HIF1a knockdown (using HIF siRNA) on PRH mRNA. If there is no change in mRNA levels in either of these conditions then we will proceed to examine PRH protein levels, PRH subcellular localisation and PRH activity under hypoxia and normoxia. The effects of HIF activity and hypoxia on a variety of PRH repressed genes will be assessed and chromatin Immunoprecipitation experiments will be carried out to examine whether PRH repression is lost under hypoxic conditions .
Increased PRH decreases tumour cells migration. We will also determine whether hypoxia blocks the effect of PRH on tumour cell migration using transwell migration assays. Cell migration assays will be carried out in a variety of tumour cell lines.

Refs: Noy et al 2010 Mol. Cell Biol. Mol Cell Biol. 2010 May;30(9):2120-34.

Soufi and Jayaraman. Biochem J. 2008 Jun 15;412(3):399-413.

How are you planning to ensure adequate supervision?

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.

Primary Supervisor:	Mark Drayson / Farhat Khanim/Chris Bunce
Project Title:	New use of old drugs in the treatment of myeloma; investigation of mechanisms of action against the malignant clone
Department:	Department of Immunity and Infection and School of Biosciences
Cancer:	Email: f.l.khanim@bham.ac.uk Tel: 0121 414 8680
Discipline:	Cancer Sciences ■ Pathology ☐ Metabolic Medicine ☐ Haematology ■ Infection ☐ Immunology ☐ Anatomy ☐ Endocrinology ☐ Liver & GI Medicine ☐ Evolutionary basis of clinical medicine ☐

Project Outline

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. Affordable, non-toxic treatments that are suitable for even elderly myeloma patients are needed urgently.

Translational research in Birmingham has identified new molecular targets for a combination of two old drugs (progesterone and bezafibrate). As a result, the drug combination is in clinical trials in the UK for adult acute leukaemia and lymphomas, and also in childhood Burkitt's lymphoma in Africa. Utilising a similar drug redeployment strategy, investigation of a library of a hundred existing drugs with diverse clinical use has identified drug combinations (an anti-tapeworm drug and an anti-epileptic) with activities against myeloma cell lines and primary tumour cells from myeloma patients bone marrow. Clinical tests are currently planned and being undertaken here in the UK and in the US with one of these agents. Three consecutive intercalating students have taken the project to the point where we have a good idea of the mode of action of at least one of the drugs. We still need to understand the mode of action of the second drug against myeloma cells. In continuing studies, we would like to understand how the drugs are affecting the mitochondria of the myeloma cells (fission and fusion), the signalling that arises as a result of mitochondrial changes, and the actions of the drugs when myeloma cells are placed in the context of other bone marrow cells and conditions such as hypoxia.

The drugs will be tested for their affect on cell proliferation, survival / apoptosis /autophagy and secretion of M-protein. Investigation into mechanisms of action and molecular targets will include measurement of reactive oxygen species, activation of nuclear transcription factors and cell signalling important in myeloma. An important aspect that will be investigated is how the drugs modify the structure and function of mitochondria and how this relates to the anti-myeloma activity of the drugs. This will be investigated using advanced fluorescence/time-lapse microscopy, immunoblotting and other molecular biology techniques.

How are you planning to ensure adequate supervision?

The student will be co-supervised by Professor Mark Drayson, Dr Farhat Khanim and Prof Chris Bunce. We will have regular meetings to discuss progress and to address any developments in the project. Daily laboratory supervision will be undertaken by Dr Farhat Khanim. Some of the techniques used in the project are undertaken in the Clinical Immunology Service run by Prof Mark Drayson. This will give the student a very useful insight into clinical laboratory techniques to compliment the cell and molecular techniques they will learn in the research laboratory. .
The student role.
Clinical researchers are absolutely essential for successful translation of research findings into the clinic. Hence it is important that intercalating students have a positive research project experience and leave with the motivation and enthusiasm to continue with research. Hence, the student will work within the supportive network of a larger group of post-docs and students, will be encouraged to interact with them, and will be treated as a member of the research team. The project is organised such that the student will be very closely supervised during the first few weeks and semester. Once the student is confident in the lab and with the laboratory protocols, the students are given more independence to organise their own time and design the experiments. The student will be encouraged to read papers and develop their own ideas and hypotheses about the work and design their own experiments. They will also be encouraged to attend national and international meetings to present their work if a relevant meeting/conference comes up. This supervisory approach has been extremely successful with our previous students. Blair Merrick won a poster prize at the International Myeloma Workshop in Paris 2011 and Hannah Giles presented the work at a national student conference 2010 and won third prize for best oral presentation. Our goal is to give the students real experience of research and the importance of the interface between the lab and the clinic.

Primary Supervisor:	Dr Steven Lee (with Prof R. Bicknell)
Project Title:	Targeting the tumour vasculature with genetically modified immune cells.
Department:	School of Cancer Sciences
Discipline:	Cancer Sciences ■ Pathology ☐ Metabolic Medicine ☐ Haematology ☐ Infection ☐ Immunology ■ Anatomy ☐ Endocrinology ☐ Liver & GI Medicine ☐ Evolutionary basis of clinical medicine ☐

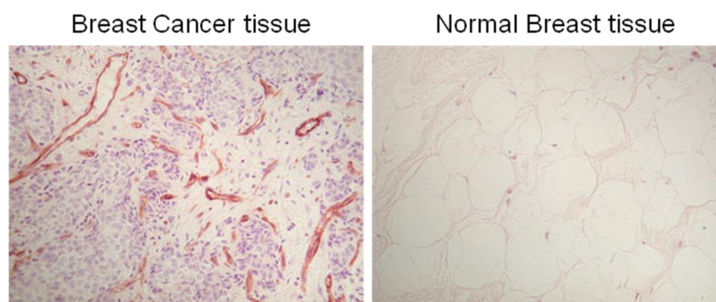
Project Outline

Compared to normal tissue, angiogenesis in tumours is deregulated and/or aberrant, resulting in a structurally and functionally abnormal vasculature. Targeting unique features of the tumour vasculature to compromise blood flow in tumour tissue should therefore provide therapeutic benefit. Anti-angiogenic monoclonal antibodies or small molecules that target these tumour endothelial markers appears to have limited curative potential, possibly because of their cytostatic action and the redundancy of angiogenic pathways. In principle, cytotoxic strategies should be more effective because they could prevent formation of new vessels and destroy existing tumour vasculature.

T lymphocytes are self-replicating effectors that can persist for years and display potent and specific cytotoxic activity. Recent clinical studies infusing cytotoxic T lymphocytes (CTLs) specific for antigens expressed on malignant cells have demonstrated remarkable efficacy in treating metastatic melanoma[1]. However, extending this therapy to other cancers is limited by a lack of appropriate tumour antigens. Targeting T cells to tumour endothelial markers offers an alternative approach that in animal models has been shown to inhibit tumour growth and prolong host survival[2,3].

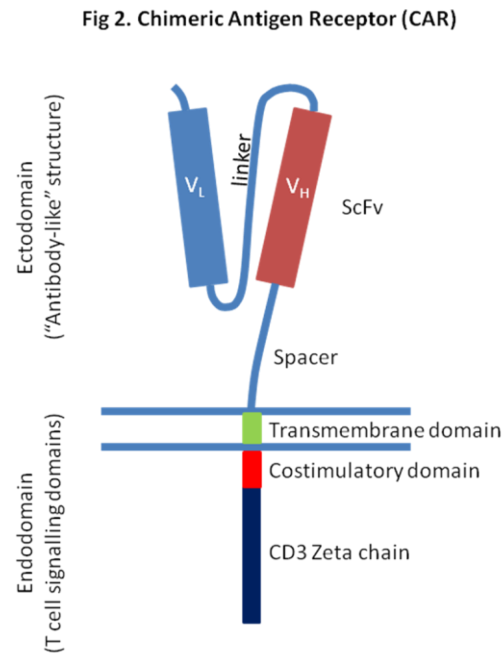
Our recent work has identified a novel tumour endothelial marker, CLEC14A[4]. This C-type lectin is highly expressed on the surface of endothelial cells lining the vasculature of many common human cancers (including breast, prostate, bladder and ovarian carcinomas) but in the vasculature of healthy tissue expression is low or undetectable (Fig. 1).

Fig. 1 CLEC14A is strongly expressed on blood vessels within breast cancer tissue but not in normal breast tissue.



It is now possible to engineer T cells with a defined specificity by transducing genes encoding so-called “chimeric antigen receptors” (CARs). CARs combine the specificity

of antibodies with the cytotoxic and immunomodulatory functions of T cells and function in an MHC-unrestricted manner (reviewed[5]). Typically, CARs consist of a single chain variable fragment (scFv) of a specific antibody linked to intracellular T cell signalling domains (Fig. 2).



The anti-tumour effects of CAR-expressing T cells have been demonstrated in pre-clinical models[6] and more recently in clinical trials[7,8]. Using a panel of novel monoclonal antibodies specific for human CLEC14A, we plan to generate CAR constructs that will target T cells to recognise and destroy the tumour vasculature.

Aims: Generation and characterisation of CLEC14A-specific CARs to assess their therapeutic potential for cancer.

The project will focus on the following three areas:

1. Using PCR, single chain variable fragment (scFv) genes will be isolated from the hybridomas that express CLEC14A-specific antibodies. These will be cloned into appropriate expression vectors for sequencing and testing for CLEC14A-specific binding.

2. The scFv gene will then be cloned into an existing retroviral expression plasmid designed to generate the CAR construct. Human T cells will be transduced in vitro using this retrovirus to stably express the CAR on the cell surface. Expression of the CAR will be explored using flow cytometry and the specificity and function of CAR-expressing T cells will be tested using several assays of T cell function exploring their ability to proliferate and release cytokines in response to CLEC14A and their ability to kill CLEC14A-expressing target cells.

3. Further studies will explore the optimal CAR design, incorporating different costimulatory domains and spacer regions to maximise T cell responses in vitro and thereby the therapeutic potential of such T cells.

This project is based on a joint study between two labs in the medical school, combining the expertise of Dr Steve Lee (T cell therapy including engineering T cells to express cloned antigen receptors) and Prof Roy Bicknell (angiogenesis).

References

1. Rosenberg, SA et al. Clin Cancer Res, (2011).
2. Chinnasamy, D et al. J Clin Invest **120**, 3953, (2010).
3. Niederman, TM et al. Proc Natl Acad Sci U S A **99**, 7009, (2002).
4. Mura, M et al. Oncogene, in press, (2011).
5. Sadelain, M et al. Curr Opin Immunol **21**, 215, (2009).
6. Brentjens, RJ et al. Nat Med **9**, 279, (2003).
7. Porter, DL et al. N Engl J Med **365**, 725, (2011).
8. Pule, MA et al. Nat.Med. **14**, 1264, (2008).

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 Bicknell labs. 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 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.

<table>
<tr><td>Primary Supervisors:</td><td>Kismet Hossain-Ibrahim
Prof. Garth Cruickshank
Prof. Ann Logan</td></tr>
<tr><td>Project Title:</td><td>Elucidating the mechanism of action of Decorin in abrogating human glioma cell lines</td></tr>
<tr><td>Department:</td><td>School of Cancer Sciences</td></tr>
<tr><td>Contact:</td><td>Email: a.logan@bham.ac.uk
Tel: 0121 414 8854</td></tr>
<tr><td>Discipline:</td><td>Cancer Sciences [x]
Pathology [ ]
Metabolic Medicine [ ]
Haematology [ ]
Infection [ ]
Immunology [ ]
Anatomy [ ]
Endocrinology [ ]
Liver & GI Medicine [ ]
Evolutionary basis of clinical medicine [ ]</td></tr>
<tr><td colspan="2">Project Outline</td></tr>
<tr><td colspan="2">Objectives:
Glioblastoma multiforme (GBM) is the most aggressive primary brain tumour. Human recombinant Decorin - a glycoprotein that modulates Transforming Growth Factor Beta (TGFβ), vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) - may suppress GBM growth. We aim to show that Decorin is the only GCP-grade drug able to suppress glioma cell growth by suppressing multiple cancer-growth pathways.

Design:
Growth rates of in vitro GBM cell lines will be determined with addition of Decorin or medium alone (control). Separate assays will add combinations of protein kinase inhibitors to inhibit TGBβ, VEGF and EGFR pathways in order to elucidate the mechanism of action of Decorin. Furthermore, we will be able to compare the efficacy of Decorin to existing commercial single-mechanism anti-cancer drugs, such as Bevacizumab (VEGF inhibitor), allowing testing in mouse model of glioma and thence a Phase 1 clinical trial

Methods:
C6, U87 and primary human (grown from patients) GBM cells will be plated at a density of 200,000/ml in complete medium. Serum-free medium containing Decorin will be administered to cells by either single doses or repeated doses of 100ug/ml/24hr. Control groups will contain serum free medium only. Daily cell counts will be analysed with T-Tests. The same experimental protocol will be followed with the addition of protein kinase inhibitors targeting the aforementioned three growth receptors.

If successful, this project will demonstrate, for the first time, a combinatorial role for decorin in treating human glioblastoma multiforme.</td></tr>
<tr><td colspan="2">How are you planning to ensure adequate supervision?</td></tr>
</table>

Kismet Hossain-Ibrahim has completed his SpR training in neurosurgery in the West Midlands and has funding to start a post-CCT Fellowship in neurosurgery with 50% research activity UOB. He currently co-supervises a full-time PhD student – Lisa Hill - with Ann Logan who is studying the effects of Decorin on gliomas. He will therefore be available two days per week to offer advice on project design and implementation with practical assistance form Lisa Hill.

Prof. Logan and Prof. Cruickshank will offer a mentorship role with the neuroscientific experience and offer intellectual drive for the project, with a weekly lab, meeting to ensure delivery of objectives with the intercalated BMedSc student.

The student role.

The student will learn become a member of Professor Ann Logan's team and contribute to weekly meetings, where he / she will be able to have daily tutelage from Lisa Hill (who will be performing similar experiments *in vivo).* The student will become proficient in techniques of:

- *in vitro* cell culture
- collection and preparation of primary tumour samples
- common cancer cell pathways
- ELISA
- Database management
- Time management
- Scientific literature retrieval and review
- Scientific paper writing

The student will be encouraged to join Prof Cruickshank and Mr. Hossain-Ibrahim in neurosurgical theatre in order to collect tumour specimens and transport tissue to the laboratory for culture. Ethical consent for use of tissue for research will be obtained by the neurosurgical team.

It is hoped that a successful student would present results of these experiments locally first and then to a National Neurosurgery and / or Neuro-oncology meeting and contribute to a publication by the end of the 2012 academic year.

Primary Supervisor:	Dr Heather Long
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 Tel: 0121 414 2808
Discipline:	Cancer Sciences [x] Pathology [ ] Metabolic Medicine [ ] Haematology [ ] Infection [ ] Immunology [x] Anatomy [ ] Endocrinology [ ] Liver & GI Medicine [ ] Evolutionary basis of clinical medicine [ ]
Contact:	Email: longhm@bham.ac.uk Tel: 0121 414 2808

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:

Long HM *et al* (2011) Cytotoxic CD4+ T cell responses to Epstein-Barr virus contrast with CD8 responses in breadth of lytic cycle antigen choice and in lytic cycle recognition. *J Immunol.* 187:92-101.

Haque T *et al* (2007). Allogeneic cytotoxic T-cell therapy for EBV-positive posttransplantation lymphoproliferative disease: results of a phase 2 multicenter clinical trial. *Blood* 110:1123-1131.

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 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 Prof A. Rickinson who has a direct interest in this work.

The student role.

The student will work alongside Dr Heather Long 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.

Primary Supervisor:	Jo Morris
Project Title:	BRCA1: from Man to Mouse and Back Again.
Department:	School of Cancer Sciences
Contact:	Email: j.morris.3@bham.ac.uk Tel: 0121 414 3016
Discipline:	Cancer Sciences X Pathology ☐ Metabolic Medicine ☐ Haematology ☐ Infection ☐ Immunology ☐ Anatomy ☐ Endocrinology ☐ Liver & GI Medicine ☐ Evolutionary basis of clinical medicine ☐

Project Outline

Inheritance of a mutation in the *BRCA1* gene contributes a very high risk of breast and ovarian cancer development. There are two recognised structures of the BRCA1 protein known to relate to the risk and gene changes that result in an amino acid substitution in these regions are related to risk of disease. At the C-terminus substitutions in the BRCT domains cause an inability of BRCA1 to recruit to sites of DNA damage and interact with proteins involved in DNA repair. Substitutions in the other region, the N-terminal RING domain is associated with an increased risk of disease. These appear to alter the ability of the protein to act as an E3 ubiquitin ligase. The link between the ligase and the activity of the protein remains controversial.

We have recently generated a mouse which carries a copy of the *Brca1* gene with a single nucleotide change that results in an amino acid change in the RING region. In humans this small change is associated with cancer predisposition. When these mice were crossed with others that were engineered to lose the remaining (good) allele of *Brca1* the mice got tumours. Unexpectedly the tumours were not sensitive to treatments that are expected to kill Brca1-deficent tumours. (Reference: Cancer Cell, December 13th 2011. 20, 797–809, DOI 10.1016/j.ccr.2011.11.014)

In other work we have tested an *in vitro* system for the interactions that the BRCA1 N-terminus makes with various proteins, including those that allow it to act as an E3 ligase. In fact we have generated over a hundred mutations in this region and mapped BRCA1 against all known proteins that it interacts with. We have discovered amino acid substitutions that inhibit interactions and, surprisingly, those that increase protein interactions. This work has given us a very clear and detailed view of how 'sensitive' the human BRCA1 RING region is to small changes.

The research project will 'make' the mouse version of the Brca1 N-terminus and its interacting proteins in our *in vitro* system which is a yeast-two-hybrid interaction analysis. The project will require the generation of these new reagents and, importantly the testing of BRCA1 mutations in the mouse version of the proteins. You will use the reagents you have made to find out whether changes in the mouse version of the gene behave in the same way as the human version that we have already mapped.

This is really important because decisions will be made about the way that patients

are going to be treated based on our understanding of the gene gleaned from mice. We need to be sure that the mouse and human proteins behave the same way before that extrapolation can be properly made.

This project has real-life implications and will be part of the laboratory's investigation into the role of genetics in breast and ovarian cancer predisposition.

How are you planning to ensure adequate supervision?

This project is an important part of the Morris lab' out-put, complementing an ongoing research project. The project will be a genuine research project and as such will be supported not only by the supervisor, but also by other members of the Morris lab'. A successful outcome of the project is anticipated to form part of published work. As a member of the lab' the student will have weekly one-to-one meetings with Dr Morris to assess progress, discuss project planning and ensure proper reporting and write up.

In a period of 8 months the student would be expected to give around three lab' meeting presentations to the 8-team group, several of which have expertise and all of which have interest in the success of the project. The student will be part of the laboratory, a vibrant and enthusiastic group of scientists interested in the role of the BRCA1 and its interacting proteins in the life of a cell, in DNA repair and in cancer development.

The student role.

The student's role is split into several portions: as a learner, to learn, on the job, important research techniques, to understand and apply principles of a scientist, to think about and design experiments (with supervision), to undertake the laboratory work with care precision and integrity, to write up results in a professional manner understandable by professionals and non-professionals alike.

This project will include several techniques in common usage in molecular biology

laboratories such as cloning, site directed mutagenesis and western blotting as well as the more specialist technique of yeast-two-hybrid analysis.

This will be an intense and, for a student prepared to put in a good deal of hard work, a rewarding research project. It would suit someone interested in a career in academic research and make an excellent introduction to anyone considering undertaking a PhD.

Primary Supervisor:	Karl Nightingale (& Bryan Turner)
Project Title:	Characterising the transcriptional response to HDAC inhibitors.
Department:	School of Cancer Sciences
Contact:	Email: k.p.nightingale@bham.ac.uk Tel: 0121 414 6833
Discipline:	Cancer Sciences x Pathology Metabolic Medicine Haematology Infection Immunology Anatomy Endocrinology Liver & GI Medicine Evolutionary basis of clinical medicine

Project Outline

Histone deacetylase inhibitors (HDACi) are a promising class of chemotherapeutic agents, two of which, SAHA ('*Vorinostat*') and depsipeptide ('*Romidepsin*'), have recently been FDA approved for the treatment of cutaneous T-cell lymphoma. However, attempts to extend the use of HDACi to other cancers have been disappointing. The main barrier to improving the clinical effectiveness of HDACi is that their mechanism(s) of action are complex and not well understood. This is primarily because there are several structural classes of HDACi, and it is not clear which are the key HDACi targets, nor do we understand the mechanisms by which HDACi affect cell function.

HDACi induce genome-wide changes in histone modifications in both tissue culture cells and in patients, and these are believed to underpin cellular responses by inducing transcriptional changes at pro-apoptotic and differentiation genes. Our recent studies on a clinically used HDACi (sodium valproate), indicate that tissue culture cells show complex transcriptional responses to treatment, with a network of chromatin-related genes responding very early after cell exposure (~30 mins treatment).

This project will build upon this work to assess whether this network of genes is (1) a common response to all HDAC inhibitors, (2) important for the cellular response(s) to these agents, and (3) shared or different in cancer cells. These studies will start to dissect the mechanisms of response to these agents, and to understand what is the clinically important aspects of their activity:

(1) Characterising transcriptional responses at 'Early response network' genes
Different HDAC inhibitors have different potencies in their abilities to induce histone acetylation and cell differentiation and apoptosis. Using concentrations of three inhibitors (TSA, SAHA, Valproate) that induce similar cellular responses in tissue culture cells, we will characterise the transcriptional changes that occur upon treatment. This study will assess transcriptional responses at these candidate genes over a time-course, and use 'wash out' experiments (i.e. where the inhibitor is removed) to identify key responses that contribute to longer-term cell differentiation and apoptosis. Later studies will use expression microarrays and bioinformatic approaches to characterise the global response.

(2) *Characterising transcriptional responses in primary tissues.*

Studies in tissue culture cells are often useful in understanding key mechanisms, but need to be validated with primary (i.e. patient-derived) cells. Key transcriptional responses identified from the tissue culture studies will be repeated with primary lymphocytes and comparable cells from patients with Burkitt's lymphoma (in collaboration with Prof. M. Rowe, Cancer Studies)

How are you planning to ensure adequate supervision?

Dr Nightingale is one of three research groups (Nightingale / O'Neill / Turner) that share lab space and focus on the contribution of histone modifications to gene regulation. This ensures that there is constant lab supervision, either by the supervisor who remains an active researcher, or by post-doctoral or PhD students.

Dr Nightingale has a 'open door' policy for queries / discussion as they arise, and it is likely that the student will talk about progress and/or their plans every few days, however, a more formal 'progress review' will be held at the start and end of each term to discuss longer term goals.

We hold a weekly joint group meeting with other groups interested in epigenetics and aspects of development, and intercalating students will be expected to present their progress approximately once a term.

.

The student role.

This project will require a number of new approaches that will take time and skill to master, notably tissue culture, protein biochemistry and molecular biology approaches. You will have lots of day-to-day help to develop these skills.

The key things we expect are (1) an interest in the broad area of epigenetic gene regulation and (2) a commitment to develop the project to its full potential – i.e. you should be prepared to work hard and intellectually engage with your work (i.e. being able to identify key questions and design experiments to address them.)

Primary Supervisor:	Dr. Elena Odintsova and Dr. Fedor Berditchevski
Project Title:	**Investigation of the role of tetraspanins in responses of breast cancer to the drugs targeting ErbB receptors**
Department:	School of Cancer Sciences
Contact:	Email: e.odintsova@bham.ac.uk Tel: 0121 414 7458
Discipline:	Cancer Sciences x [ ] Immunology [ ] Pathology [ ] Anatomy [ ] Metabolic Medicine [ ] Endocrinology [ ] Haematology [ ] Liver & GI Medicine [ ] Infection [ ] Evolutionary basis of clinical medicine [ ]

Project Outline

Transmembrane receptors of ErbB family are important for normal development and maintenance of tissue homeostasis. However, deregulation of their activity on one hand, and elevated levels of the receptors on the other lead to the progression of many diseases including cancer. For instance, ErbB2 (HER2) receptor is overexpressed in 15-25% of breast cancers and correlates with poor prognosis. ErbB receptors are targeted by recently approved drugs such as Herceptin and Lapatinib. Unfortunately, in many cases after initial success cancer cells develop resistance to these drugs within a year. Furthermore, some patients do not respond to these drugs at all.

Research in our laboratory has shown that one of the factors affecting responses to Herceptin and Lapatinib is the assembly of tetraspanin protein complexes in breast cancer cells. Tetraspanin proteins are the organisers and structural blocks of the specialised microdomains at plasma membrane. Some of the tetraspanins were shown to associate with ErbB receptors and known to modulate their activity. The proposed project aims to investigate how tetraspanins increase sensitivity of breast cancer cells to the treatments.

The specific aims of the project are:
a) to generate breast cancer cell lines with variable expression levels of tetraspanins and ErbB receptors;
b) to examine responses of these cell lines to the drugs targeting ErbB receptors in biological assays (e.g. proliferation and migration);
c) to investigate the activity of ErbB receptors in these cell lines.

How are you planning to ensure adequate supervision?

We supervise intercalating students on a daily basis in the lab, provide training in necessary techniques and ensure adequate scientific background.   .
The student role.
The student will be trained to carry out experiments independently, to analyse the results and to discuss the outcome of the experiments and future applications of the acquired knowledge. The project is a part of the ongoing research in the laboratory, and all participants will be included in the publication of the results when the time comes.

Primary Supervisor:	Eva Petermann
Project Title:	**Role of a leukaemia oncogene in DNA replication and -damage**
Department:	School of Cancer Sciences
Contact:	e.petermann@bham.ac.uk http://www.birmingham.ac.uk/eva-petermann
Discipline:	Cancer Sciences [x] Pathology [ ] Metabolic Medicine [ ] Haematology [ ] Infection [ ] Immunology [ ] Anatomy [ ] Endocrinology [ ] Liver & GI Medicine [ ] Evolutionary basis of clinical medicine [ ]

Project Outline

Purpose

Our lab is interested in the proper control of DNA replication, which prevents DNA damage and has important roles in cancer development and –treatment. The MLL protein, which is frequently subject to oncogenic (cancer-promoting) mutations in aggressive infant leukaemias, was recently implicated in DNA replication control. This project aims to investigate whether loss of normal MLL function causes aberrant DNA replication and DNA damage that can be potentially exploited for therapeutic purpose.

Background

DNA replication is important for both cancer development and -treatment. Many oncogenes cause aberrant DNA replication and replication-associated DNA damage ("replication stress"), a proposed driving factor of genomic instability during cancer development. Replication stress is therefore cancer-promoting, but the increased DNA damage caused by it can make cells more reliant on DNA repair pathways for survival, which can be exploited for cancer treatment with DNA repair inhibitors.
The *MLL* gene is subject to oncogenic translocations in at least 10% of all human leukaemias, especially infant leukaemias. MLL was recently found to be involved in replication control, by acting downstream of the S phase checkpoint kinase ATR. ATR is activated by DNA damage during replication and suppresses further initiation of replication and promotes cell cycle arrest and DNA repair. We and others have shown that limiting replication initiation by ATR is important to prevent replication stress during the normal cell cycle and, in line with this, ATR has been found to act as a tumour suppressor.
As mutations in MLL disrupt control of replication initiation by ATR, we hypothesise that cells with such mutations might suffer from oncogenic replication stress, which could have a promoting function in leukaemia development. This hypothesis is supported by our preliminary data. Furthermore, this replication stress may render leukaemia cells lacking proper MLL more dependent on DNA repair pathways, a weakness that can be exploited for cancer therapy.

Project objectives

This project will address the following questions:
1. Does MLL dysfunction lead to replication stress and spontaneous DNA damage?
2. Do cells with MLL mutations depend on DNA repair pathways for their survival?

Scientific approach

You will investigate the above questions using mammlian cell models, WT and *Mll*$^{-/-}$ mouse embryonic fibroblasts (MEFs) and Jurkat T-lymphocytes expressing an oncogenic MLL fusion protein. Changes to patterns of DNA replication, such as initiation, progression and stalling of replication forks will be measured by pulse-labelling cycling cells with thymidine analogues, to be detected by immunofluorescence staining for replication foci and DNA fibre analysis. DNA damage will be analysed by immunofluorescence staining for nuclear foci of DNA damage markers (phospho-Serine139 H2AX (·H2AX), RPA, and 53BP1) to detect stalled replication forks and DNA breaks. To measure genomic instability, you will analyse metaphase chromosome spreads from MLL-mutated cells for gaps or breaks at common fragile sites (CFS), which are indicators of replication stress-induced genomic instability.
To test whether cells with dysfunctional MLL rely on DNA repair pathways for survival, you will use clinically relevant DNA repair inhibitors (inhibitors of Chk1 (PD-407824), PARP (AZD2281and ATM (KU55933)). You will measure the impact of these agents on survival in cells with disrupted MLL function using colony-forming (clonogenic) assays.

Expected outcomes
This work will enable you to show whether loss of MLL function causes replication stress and genomic stability, which may lead to a better understanding how infant leukaemias start and develop. The work will further help uncover proteins or pathways that cells with MLL fusions rely on for proliferation and may lead to future investigations into cancer treatment by synthetic lethality.

Key references
Liu, H., Takeda, S., Kumar, R., Westergard, T.D., Brown, E.J., Pandita, T.K., Cheng, E.H. & Hsieh, J.J. Phosphorylation of MLL by ATR is required for execution of mammalian S-phase checkpoint. *Nature* **467**, 343-6 (2010).

Petermann, E., Woodcock, M. & Helleday, T. Chk1 promotes replication fork progression by controlling replication initiation. *Proc Natl Acad Sci U S A* **107**, 16090-5 (2010).

Syljuasen, R.G., Sorensen, C.S., Hansen, L.T., Fugger, K., Lundin, C., Johansson, F., Helleday, T., Sehested, M., Lukas, J. & Bartek, J. Inhibition of human Chk1 causes increased initiation of DNA replication, phosphorylation of ATR targets, and DNA breakage. *Mol Cell Biol* **25**, 3553-62 (2005).

Dereli-Oz, A., Versini, G. & Halazonetis, T.D. Studies of genomic copy number changes in human cancers reveal signatures of DNA replication stress. *Mol Oncol* **5**, 308-14 (2011).

Helleday, T., Petermann, E., Lundin, C., Hodgson, B. & Sharma, R.A. DNA repair pathways as targets for cancer therapy. *Nat Rev Cancer* **8**, 193-204 (2008).

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, and the PI. 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, who 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 labs of Profs Tanja Stankovic and Malcolm Taylor.
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.

Primary Supervisor:	Prof. Antal Rot		
Project Title:	CCR7, CCRL1 and their ligands in tumour host interactions		
Department:	Infection and Immunity		
Contact:	Email: a.rot@bham.ac.uk Tel: 0121 414 3454		
Discipline:	Cancer Sciences X Pathology ☐ Metabolic Medicine ☐ Haematology ☐ Infection ☐	Immunology X Anatomy ☐ Endocrinology ☐ Liver & GI Medicine ☐ Evolutionary basis of clinical medicine ☐	

Project Outline

Chemokines play important multiple roles in cancer development and spread as well as host responses to cancer. Importantly, the expression of chemokine receptors by tumour cells aids their metastasis, which in the most clinical settings is the life-limiting factor of malignant diseases. It has been well known that CCR7 expression by tumour cells is associated with enhanced lymphogenic metastases in both human and experimental tumours. However, CCR7 and its ligands are also fundamentally involved in the development of anti-cancer immunity as well as its regulation by FoxP3 positive Treg cells. In our lab we use B16 *in vivo* murine melanoma model to investigate how CCR7 expression by host cells impinges on the developing anti-cancer immunity and how the expression of CCR7 and its ligands by the tumour cells may influence immune responses. However, tumour cells can also express CCRL1, an atypical chemokine receptor for both CCR7 ligands, with hitherto unknown consequences. Thus, an entirely new set of our planned studies will address the question how CCRL1 impinges on tumour-host interactions. To this end, murine melanoma tumour cell line B16, which expresses luciferase (B16luc) and thus can be imaged intravitally, will be stably transfected with CCRL1 using a retroviral vector. The resulting B16-CCRL1 cell lines will be subcutaneously inoculated into wild type and mice deficient for CCR7, CCRL1 and their ligands. Tumour growth and spread and functional parameters of anti-tumour immunity as well as immuno-morphological appearance of tumours will be compared for B16 and B16-CCRL1 using IVIS imaging, confocal microscopy and FACS, respectively. In the second stage of work B16-luc melanoma cell line will be transfected with both classical chemokine receptor CCR7 and its atypical counterpart CCRL1. This will allow us to investigate the role of CCRL1 in tumour growth and metastasis when expressed on tumour cells in cis-geometry with the pro-metastatic chemokine receptor CCR7. This project should give student good understanding of the complexities of tumour-host interactions and advance our knowledge on the chemokine-driven circuits in cancer growth and tumour immune regulation.

How are you planning to ensure adequate supervision?

I plan to monitor the progress of the student's project by meeting with the student at least twice a week. In addition. the regular Rot-lab meeting takes place once a week where each member is reporting on the experimental progress and other research-related issues are discussed. The two post-docs in the lab have volunteered and will be able to supervise the student on a daily basis.
The student role.
Student will learn the basics of in vitro tissue culture, gene cloning into viral vectors and their use in cell transfection as well as the use of PCR, ELISA, and other basic molecular and cell biology techniques. The project will require the completion by the student of the HO animal course. Subsequently, the student will learn the basics of in vivo animal manipulations, use of Intravital IVIS imaging, animal necropsy, histological tissue processing, immunofluorescent staining use of confocal microscopy and FACS.

Primary Supervisor:	Dr Andy Turnell
Project Title:	Role of the PBF oncogene in thyroid cancer pathogenesis.
Department:	School of Cancer Sciences
Contact:	a.s.turnell@bham.ac.uk
Discipline:	Cancer Sciences ■ Pathology ☐ Metabolic Medicine ☐ Haematology ☐ Infection ☐ Immunology ☐ Anatomy ☐ Endocrinology ■ Liver & GI Medicine ☐ Evolutionary basis of clinical medicine ☐

Project Outline

Pituitary tumor transforming gene (PTTG)-binding factor (PBF or PTTG1IP) is a little characterized proto-oncogene whose over-expression is implicated in the aetiology of breast, colon and thyroid tumours. We have recently determined that PBF associates with p53 in thyroid cells and represses p53 activation (Figure 1).

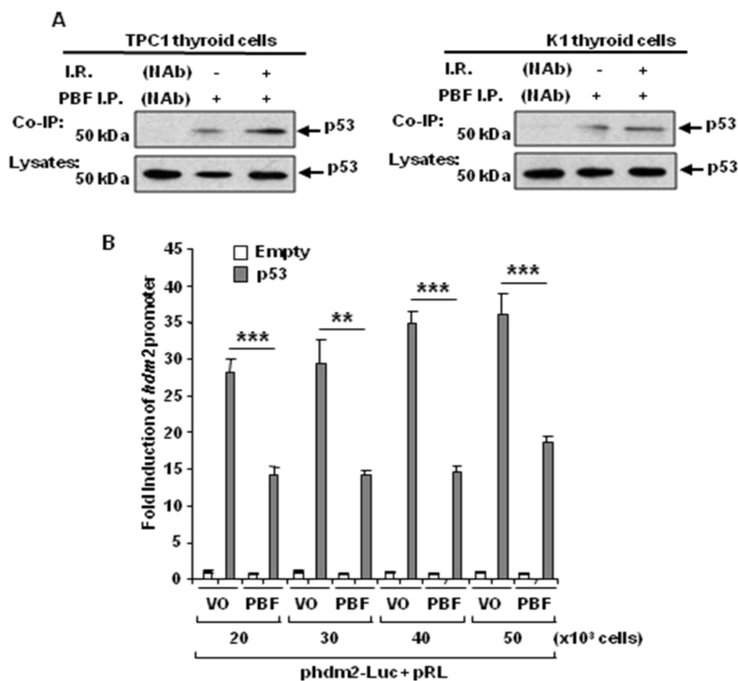


Fig. 1. PBF binds and inhibits p53 transcriptional activity. (A) Coimmunoprecipitation of endogenous p53 with an anti-PBF antibody in TPC1 and K1 cells either untreated (-) or irradiated (+). NAb: no-antibody control. (*B*) PBF inhibits p53 transcriptional activation of the Mdm2 promoter. ***, $P < 0.001$; **, $P < 0.05$.

We have also determined that PBF overexpression promotes genomic instability in a transgenic murine model (Figure 2).

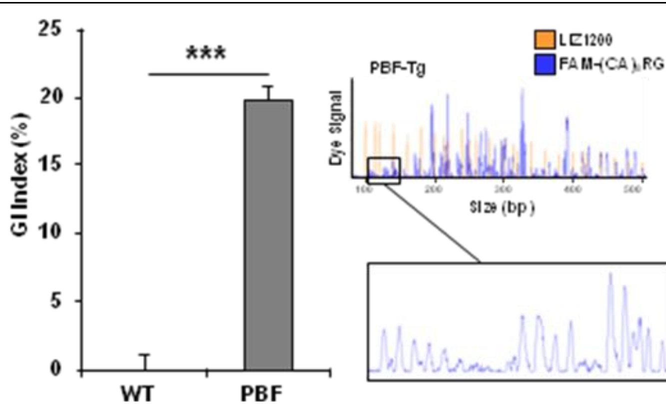


Figure 2: PBF promotes genomic instability. Genomic DNA extracted from mouse thyrocytes (n = 5) was PCR amplified using a 5′ FAM-labelled $(CA)_8$RG primer and analysed by capillary electrophoresis to generate the mean GI index value ± SEM. A representative FISSR-PCR trace amplified from PBF-Tg thyrocyte genomic DNA is shown plotted against a LIZ1200 size standard. ***, $P < 0.001$.

Given these findings we now wish to determine the molecular basis of PBF-induced genomic instability in greater detail. We hypothesize that overexpression of PBF promotes genomic instability and thyroid tumourigenesis, at least in part by suppressing DNA damage response pathways regulated by ATM, and/or ATR kinases.

During this project the student will investigate whether PBF overexpression affects the cellular response to DNA damage. Initially, the student will assess whether overexpression of PBF in thyroid cells promotes DNA damage and activates the DNA damage response pathways regulated by the ATM and ATR kinases. The student will then investigate whether PBF affects ATM or ATR activation following exposure of PBF-overexpressing thyroid cells to ionizing, or UV radiation.

Taken together, these experimental approaches will provide mechanistic insight into how PBF promotes genetic instability and thyroid tumourigenesis.

How are you planning to ensure adequate supervision?

I spend the majority of time 'at the bench' and so will be able to supervise the student closely. The project will be co-supervised by Chris McCabe in the School of Clinical and Experimental Medicine, with whom I collaborate closely. Additionally, there are post-docs and PhD students in the Turnell and McCabe labs working on related projects who will also be able to help. The student will have regular meetings with the supervisors to discuss progress, and the student will also discuss their findings at lab meetings.

The student role.

The student will investigate specifically whether overexpression of the PBF proto-oncogene product modulates DNA damage signalling pathways in thyroid cells. They will utilise cell biology, protein biochemistry and molecular biology techniques established in the laboratory to investigate whether the function of ATM or ATR kinases is affected by PBF overexpression. Results obtained by the student during this project will shed new insight into how PBF overexpression promotes thyroid tumourigenesis, It is anticipated that results from this project will form the basis of future publications.

Primary Supervisor:	Dr Doug Ward (with Drs Rik Bryan and Wenbin Wei)
Project Title:	**Identification and Evaluation of Biomarkers for the Detection of Bladder Cancer**
Department:	School of Cancer Sciences
Contact:	Email: d.g.ward@bham.ac.uk Tel: 01214149528
Discipline:	Cancer Sciences X Pathology ☐ Metabolic Medicine ☐ Haematology ☐ Infection ☐ Immunology ☐ Anatomy ☐ Endocrinology ☐ Liver & GI Medicine ☐ Evolutionary basis of 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 invasive telescope 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 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* with the aim of identifying protein biomarkers. In parallel, we are analysing DNA methylation in urine sediments with the aim of validating published epigenetic biomarkers.

Plan of investigation:

1) The proteins in the secretomes of 3 bladder cancer cell lines will be trypsinised, and the peptides analysed by LC-MS/MS.
2) Publicly available gene expression data including three mRNA expression datasets (GSE3167, GSE7476 and GSE13507) containing normal controls, non-muscle-invasive and muscle-invasive bladder cancer will be analysed.
3) Proteins showing tumour specific upregulation and detectable in secretomes will be validated by ELISA.
4) Published DNA methylation markers for bladder cancer detection will be evaluated using our large cohort of samples.
5) The sensitivity and specificity of protein and methylation biomarkers will be compared.

How are you planning to ensure adequate supervision?

The student will be supervised by Dr Doug Ward during all wet lab work and Dr Wenbin Wei will supervise bioinfomatic and statistical analyses. Dr Rik Bryan will act as 3rd supervisor and provide additional expertise in bladder cancer, especially from the clinical perspective. The student will meet with all three 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.

Primary Supervisor:	Dr. Wenbin Wei
Project Title:	Proof of principle for stratified therapy of Hodgkin's lymphoma
Department:	School of Cancer Sciences
Contact:	Email: w.wei@bham.ac.uk Tel: 0121 414 3293
Discipline:	Cancer Sciences X Pathology ☐ Metabolic Medicine ☐ Haematology ☐ Infection ☐ Immunology ☐ Anatomy ☐ Endocrinology ☐ Liver & GI Medicine ☐ Evolutionary basis of clinical medicine ☐

Project Outline

Hypothesis and aims: We hypothesize that genetic changes not only drive pathogenesis of Hodgkin's lymphoma but also provide opportunities for targeted therapy. The aim of this project is to investigate if existing drugs can target Hodgkin's lymphoma tumour cells carrying particular mutations.

Background: Hodgkin's lymphoma patients are currently treated with multi-agent chemotherapy protocols such as MOPP (mechlorethamine, vincristine, procarbazine, prednisone), ABVD (adriamycin, bleomycin, vinblastine, dacarbazine) and BEACOPPescalated (bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine, procarbazine, prednisone)[1]. However, these treatments do not take into account the genetic differences present in the malignant cells between different Hodgkin's lymphoma patients, neither do they take account of the potential to target cancer-associated mutations for the specific killing of tumour cells. Recently, we have interrogated the database of somatic mutations in cancer [2] and found that 39.3% of Hodgkin's lymphoma contains mutations in SOCS1, 31.5% in TNFAIP3, 16.7% in KDM6A, 16% in TP53, 15.6% in NRAS, 14.3% in PIK3R1, 11% in CDKN2A, 4.8% in CYLD and 2.3% in HRAS. We also found that 2% of Hodgkin's lymphoma contains NPM1/ALK gene fusion and 1.5% SEC31A/JAK gene fusion. We have searched the comprehensive drugbank database and found that there are FDA-approved drugs for PIK3R1, HRAS, JAK2 and TP53, but no FDA-approved drugs for SOCS1, TNFAIP3, KDM6A, NRAS, CDKN2A, CYLD and ALK. Because Hodgkin's lymphomas are genetically heterogeneous, we believe there is the opportunity to develop stratified therapies for this disease. This project will test the efficacy not only of existing FDA approved drugs but also identify new drugs which target SOCS1 and TNFAIP3 in Hodgkin's lymphoma.

Plan of investigation:
As the first step to do this, we propose to investigate the effects of existing drugs on Hodgkin's lymphoma cell lines with and without particular mutations. Two groups of drugs will be studied. The first group of drugs are those currently used for the treatment of Hodgkin's lymphoma, i.e. those mentioned above. The Second group of drugs are those identified through the bioinformatic analyses of the NCI-60 DTP Human Tumor Cell Line Screen data, which contains growth inhibition data of 43000 compounds on 60 human tumour cell lines and the gene expression microarray data and mutation data of 91 genes of these cell lines. We have identified potential drugs which have stronger growth inhibition effect on cell lines that have absent or low expression of target genes (SOCS1 and TNFAIP3) than those with higher expression

of these genes. We will also perform bioinformatic analysis to identify drugs that have stronger growth inhibition effect on cell lines with mutant NRAS than on those with wild type NRAS.

The student will perform experiments to investigate the effects of these drugs using cell lines with and without particular mutations. To confirm any drug effects are specific to the gene of interest, the student will also perform over-expression and knockdown studies. The sensitivity of the cell lines to drug-induced cell growth inhibition will be determined using Promega's CellTiter-Blue® Cell Viability Assay. Caspase-GLo 3/7 assay and cell viability assay will be performed as we described previously [3].

References

1. Eichenauer, D. A. and A. Engert (2011). **One size for all in Hodgkin lymphoma?** Blood **117**(9): 2557-2558.
2. Forbes et al. (2011) **COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer.** Nucl. Acids Res. **39** (suppl 1): D945-D950.
3. Lu, X., W. Wei, J. Fenton, M. S. Nahorski, E. Rabai, A. Reiman, L. Seabra, Z. Nagy, F. Latif and E. R. Maher (2011). **Therapeutic targeting the loss of the birt-hogg-dube suppressor gene**. Mol Cancer Ther **10**(1): 80-9.

How are you planning to ensure adequate supervision?

In addition to Dr. Wenbin Wei, Prof. Paul Murray and Dr. Xiaohong Lu will also co-supervise the student. The supervisors will meet with the students regularly to ensure progress, direct reading and provide support in the acquisition of necessary laboratory skills, solve any issues in a timely fashion and provide support in the writing of the dissertation.

The student role.

The student will read relevant references, learn necessary laboratory skills, perform experiments, analyze data and discuss with the supervisors on any issues relevant to this project.

Primary Supervisor:	Supervisor: Professor Keith Wheatley. Methodology support: Jayne Wilson. Clinical support: Dr Pam Kearns.
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
Contact:	Email: j.s.wilson.1@bham.ac.uk Tel: 0121 414 3461
Discipline:	Cancer Sciences ■ Immunology ☐ Pathology ☐ Anatomy ☐ Metabolic Medicine ☐ Endocrinology ☐ Haematology ☐ Liver & GI Medicine ☐ Infection ☐ Evolutionary basis of clinical medicine ☐

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.

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.

Primary Supervisor:	Supervisor: Jayne Wilson Methodology support: Professor Keith Wheatley. Clinical support: Dr Pam Kearns.
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
Cancer:	Email: j.s.wilson.1@bham.ac.uk Tel: 0121 414 3461
Discipline:	Cancer Sciences ■ Pathology ☐ Metabolic Medicine ☐ Haematology ☐ Infection ☐ Immunology ☐ Anatomy ☐ Endocrinology ☐ Liver & GI Medicine ☐ Evolutionary basis of clinical medicine ☐

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.

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.

Primary Supervisor:	Mark Cobbold and David Millar
Project Title:	Antibody Engineering to Better Target Cancer.
Department:	School of Immunity and Infection
Contact:	Email: m.cobbold@bham.ac.uk Tel: 0121 414 6839
Discipline:	Cancer Sciences [X] Pathology [ ] Metabolic Medicine [ ] Haematology [ ] **Infection** [ ] Immunology [X] Anatomy [ ] Endocrinology [ ] Liver & GI Medicine [ ] Evolutionary basis of clinical medicine [ ]

Project Outline

Therapeutic antibodies have emerged as an important class of biological targeted agents with considerable application in the management of patients with cancer. However, to date, these agents do not typically offer cancer patients curative treatment options and frequently offer only modest benefit in survival.

Therapeutic antibodies function by either blocking growth factor signals, or by engaging cytotoxic effector mechanisms of the innate immune response such as complement and phagocyte activation. Tumours are able to eventually evade these mechanisms leading to clinical relapse. Indeed, for most tumour-targeting therapeutic antibodies, only very marginal benefit is observed. Importantly, the most potent cytotoxic effector immune response lies within our adaptive immunity – the cytotoxic T-lymphocyte - yet antibodies are not able to engage with this effector cell.

Recently, we have developed a new technology that allows T cells to indirectly engage with antibodies. The antibody-peptide conjugate consists of virus derived peptide directly conjugated to an antibody. The peptide is delivered to a target cell and loaded onto MHC class-I or Class-II molecules on the cell surface. This promotes T cell receptor mediated recognition of the target cell by virus-specific Cytotoxic T Lymphocytes (CTL) and subsequent re-directed killing. Furthermore, we have shown this novel approach is highly effective in vitro but also in animal studies of human cancer.

The aim of this project will be to extend these studies into novel therapeutic antibodies. P-cadherin and E-cadherin are calcium dependent cell adhesion proteins that are over expressed on a large number of solid malignancies. Antibodies targeting these are currently in clinical trials. In this project the student will re-engineer anti-P-cadherin and anti-E-cadherin and assess the potential of these to target a number of tumour types both *in vitro* and *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 David Millar. Secondly, day-to-day supervision will be provided by David Millar to ensure the progression of the project. A number of PhD students are also working on related projects in the laboratory and thus the student will be adequately supported.

.

The student role.

The role of the student will be to work closely alongside Dr Millar in developing the antibody-peptide conjugates and also designing a novel single-chain antibody *in silico* that will be expressed and tested.
Therapeutic antibodies will be provided by Pfizer and modified by the student. Mice will be given human tumours and human killer T cells with or without antibodies and the ability of the modified antibodies to control tumour outgrowth determined.

The student will gain skill in antibody engineering, in vitro tumour cytotoxicity studies and preclinical animal models of human malignant disease.

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 XX Haematology ☐ Infection ☐ Immunology ☐ Anatomy ☐ Endocrinology ☐ Liver & GI Medicine ☐ Evolutionary basis of clinical medicine XX

Project Outline

The nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR·) 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··plays multiple roles in lipid physiology and when misregulated lead to various diseases associated with fat storage.
Understanding the molecular mechanisms of PPAR· function may lead to identification of drug targets and development of novel drugs. Recent data in various model systems suggest signals mediated by PPAR· are transduced through specific 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 initation 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 PPARg signaling [3]. We have shown that PPAR· binds TAF8, and that TAF8 is required for lipogenesis in zebrafish embryos. In this project, we will modulate PPAR signaling by antagonists and agonists 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· 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**, 533-540, (2010).
2 Lieschke, G. J. & Currie, P. D. Animal models of human disease: zebrafish swim into view. *Nat Rev Genet* **8**, 353-367 (2007).
3 Guermah, M., Ge, K., Chiang, C. M. & Roeder, R. G. The TBN protein, which is essential for early embryonic mouse development, is an inducible TAFII implicated in adipogenesis. *Mol Cell* **12**, 991-1001, (2003).

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.

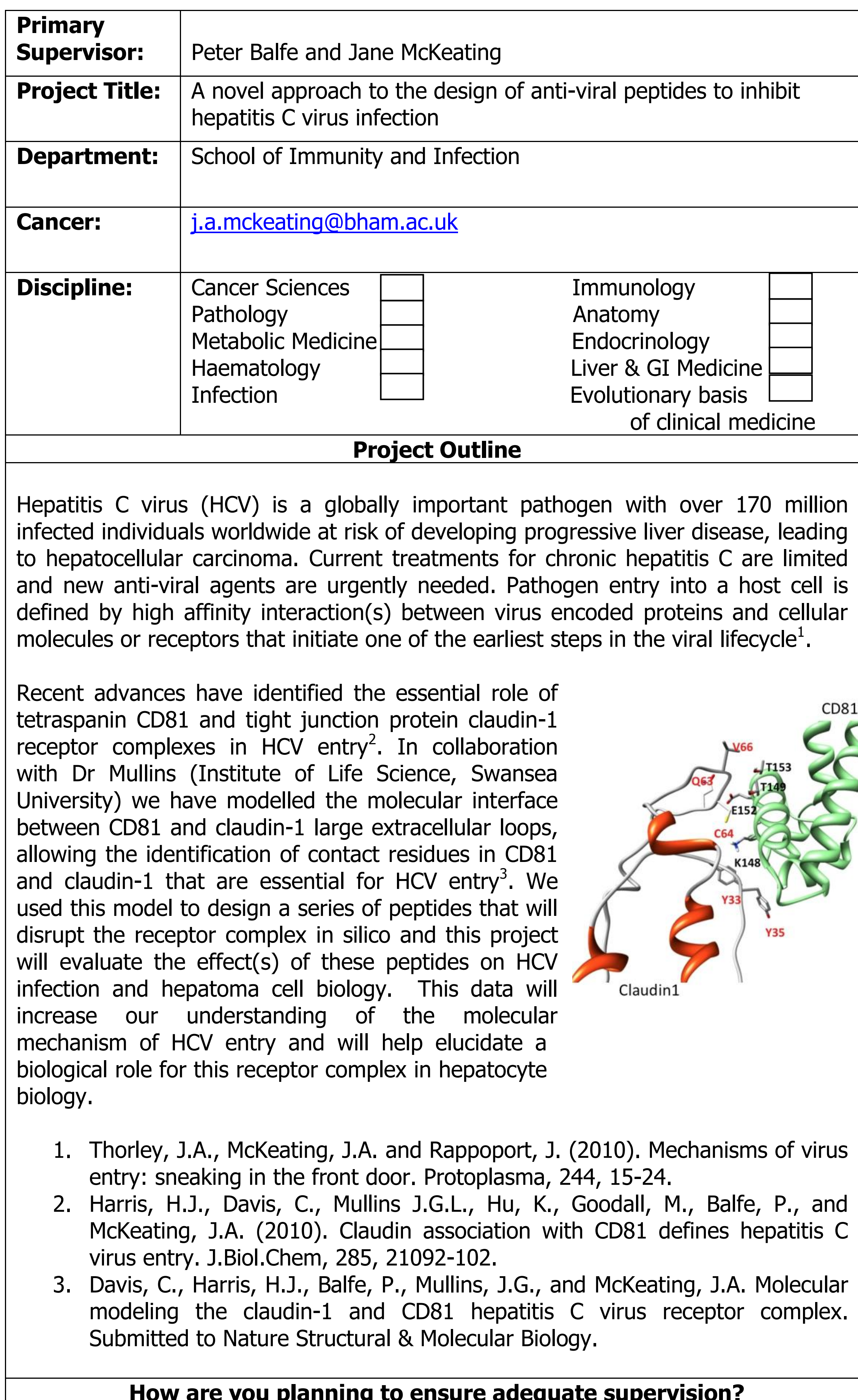
To train in the experimentel techniques required fro the project, to carry out experiments designed together with supervisors and to give oral progress reports in labmeetings aided by ppt presentations. To write a thesis and generate figures that may be suitable for use in a planned publication which may benefit from the outlined project.

Primary Supervisor:	Peter Balfe and Jane McKeating
Project Title:	A novel approach to the design of anti-viral peptides to inhibit hepatitis C virus infection
Department:	School of Immunity and Infection
Cancer:	j.a.mckeating@bham.ac.uk
Discipline:	Cancer Sciences ☐ Pathology ☐ Metabolic Medicine ☐ Haematology ☐ Infection ☐ Immunology ☐ Anatomy ☐ Endocrinology ☐ Liver & GI Medicine ☐ Evolutionary basis of clinical medicine ☐

Project Outline

Hepatitis C virus (HCV) is a globally important pathogen with over 170 million infected individuals worldwide at risk of developing progressive liver disease, leading to hepatocellular carcinoma. Current treatments for chronic hepatitis C are limited and new anti-viral agents are urgently needed. Pathogen entry into a host cell is defined by high affinity interaction(s) between virus encoded proteins and cellular molecules or receptors that initiate one of the earliest steps in the viral lifecycle[1].

Recent advances have identified the essential role of tetraspanin CD81 and tight junction protein claudin-1 receptor complexes in HCV entry[2]. In collaboration with Dr Mullins (Institute of Life Science, Swansea University) we have modelled the molecular interface between CD81 and claudin-1 large extracellular loops, allowing the identification of contact residues in CD81 and claudin-1 that are essential for HCV entry[3]. We used this model to design a series of peptides that will disrupt the receptor complex in silico and this project will evaluate the effect(s) of these peptides on HCV infection and hepatoma cell biology. This data will increase our understanding of the molecular mechanism of HCV entry and will help elucidate a biological role for this receptor complex in hepatocyte biology.



1. Thorley, J.A., McKeating, J.A. and Rappoport, J. (2010). Mechanisms of virus entry: sneaking in the front door. Protoplasma, 244, 15-24.
2. Harris, H.J., Davis, C., Mullins J.G.L., Hu, K., Goodall, M., Balfe, P., and McKeating, J.A. (2010). Claudin association with CD81 defines hepatitis C virus entry. J.Biol.Chem, 285, 21092-102.
3. Davis, C., Harris, H.J., Balfe, P., Mullins, J.G., and McKeating, J.A. Molecular modeling the claudin-1 and CD81 hepatitis C virus receptor complex. Submitted to Nature Structural & Molecular Biology.

How are you planning to ensure adequate supervision?

All students in the McKeating laboratory are assigned a post-doctoral scientist to provide training and supervision on the laboratory aspects of the project. The student and Fellow meet with Prof. McKeating weekly to review data and to plan experimental objectives. In addition to this one-to-one mentoring scheme the student will join a vibrant laboratory of basic and clinical scientists who work in a collaborative manner to study liver disease as evidenced by the labs publication record (**See web links:** HCV Group:School of Immunity and Infection). The HCV research lab holds weekly lab meeting and journal clubs, allowing the student to interact with a wide group of enthusiastic scientists.

The student role.

The student will be trained in cell culture, virus propagation and high resolution imaging of CD81 and claudin-1 trafficking, including Fluorescent Recovery After Photobleaching (FRAP) and Fluoresecent Resonance Energy Transfer (FRET). The peptide data will form part of a larger study that will be submitted for publication in a high impact journal. These techniques provide a generic skill base that is widely applicable to a range of biologically diverse projects.

<table>
<tr><td>Primary Supervisor:</td><td>Jill Brooks</td></tr>
<tr><td>Project Title:</td><td>Epstein-Barr virus-induced T cell responses and the pathogenesis of Multiple Sclerosis</td></tr>
<tr><td>Department:</td><td>School of Cancer Sciences</td></tr>
<tr><td>Contact:</td><td>j.m.brookes@bham.ac.uk</td></tr>
<tr><td>Discipline:</td><td>Cancer Sciences ☐
Pathology ☐
Metabolic Medicine ☐
Haematology ☐
Infection ■
Immunology ■
Anatomy ☐
Endocrinology ☐
Liver & GI Medicine ☐
Evolutionary basis of clinical medicine ☐</td></tr>
<tr><td colspan="2">Project Outline</td></tr>
<tr><td colspan="2">Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) whose pathogenesis involves both genetic and environmental factors. While the genetic factors increasingly implicate genes regulating immune responses, the identity of the environmental factors remains controversial. However, increasingly strong epidemiologic and laboratory findings implicate Epstein-Barr virus (EBV), a common human herpesvirus, in disease pathogenesis.

The project will explore the hypothesis that EBV infection plays a role in MS pathogenesis by inducing CD8+ and/or CD4+ T cell responses that cross-react with peptide epitopes derived from self-proteins expressed in the CNS. Whilst EBV-induced T cell responses principally comprise T cells recognising epitopes derived from viral proteins, we are also interested in a recently characterised subset of CD4+ T cells recognising cellular proteins whose expression is up-regulated in B cells by EBV infection. Initial experiments will compare and contrast T cell responses between four donor groups (i) MS patients at first diagnosis, (ii) patients with established relapsing-remitting MS, (iii) healthy controls with a recent history of symptomatic primary EBV infection (infectious mononucleosis, IM) which itself predisposes to MS and (iv) healthy EBV-immune donors with no history of IM. Based on preliminary results a range of responses will be selected for more detailed analysis, this will include T cell cloning, identification of cross-reactive potential and phenotypic/functional characterisation.</td></tr>
<tr><td colspan="2">How are you planning to ensure adequate supervision?</td></tr>
</table>

Jill Brooks works in the laboratory on a very regular basis and will be able to teach the student all the required practical techniques as well as providing help with background, planning, data analysis, project development etc. The student will be part of a larger research group and will attend weekly group meetings. Collectively group members have a very broad range of expertise and will actively support the student in all areas of the project.

.

The student role.

The project will form part of a recently established study into the potential role of EBV in the pathogenesis of MS. The student role will include the following:

(i) Generation of polyclonal/clonal T cells, specific for EBV antigens and/or cellular proteins whose expression is unregulated by EBV infection of B cells, from the blood of MS patients and healthy control donors

(ii) Screening of these T cell populations for cross-reactive potential using recombinant vaccinia viruses expressing individual CNS proteins and peptide libraries

(iii) Characterisation of any cross-reactive T cell clones (HLA restriction, functional avidity, etc.)

(iv) Analysis of the frequency/phenotype of cross-reactive responses in the blood of MS patients versus healthy control donors

Laboratory techniques to be used will include tissue culture, cytokine ELISAs and flow cytometry. In addition, the student will be actively involved in the on-going development of the project dependent on results obtained.

Primary Supervisor:	Kai-Michael Toellner
Project Title:	Role of IgG1 in immunity to Salmonella infection
Department:	School of Immunity and Infection
Contact:	Tel: 0121 415 8687 Email: k.m.toellner@bham.ac.uk
Discipline:	Cancer Sciences ☐ Immunology ■ Pathology ☐ Anatomy ☐ Metabolic Medicine ☐ Endocrinology ☐ Haematology ☐ Liver & GI Medicine ☐ Infection ■ Evolutionary basis of clinical medicine ☐

Project Outline

Salmonella infection is a major health problem in infants in subsaharan Africa. We are studying the role of vaccination induced antibodies for immunity to Salmonella in mice. While innate immunity and T cells are important in the early stages of Salmonella infection, antibodies can be protective during reinfection and recall responses. Although Salmonella induces a Th1 type T cell response, which in mice induces immunoglobulin class switching to IgG2a in B cells, we have shown recently that IgG1 antibodies provide far better protection from Salmonella infection. The mechanism of action of IgG1 is not clear. IgG1 does not bind preferentially to any of the known Fcγ receptors, and does not fix complement better than other IgG classes. We plan to study the action of Salmonella-specific IgG1 *in vitro* and *in vivo*. *In vitro* macrophages will be incubated with green fluorescent protein (GFP) transfected Salmonella opsonised with different classes of Salmonella specific Ig. Attachment, uptake and killing of fluoresent Salmonella by macrophages will be studied by confocal microscopy and cell counts of intra- and extracellular surviving bacteria. Similar experiments will be done *in vivo* after short term infection with opsonized Salomenella to study which types of cells absorb and neutralize Salmonella in different locations such as blood, peritoneum, gut and liver. Cells carrying fluorescent material from GFP transfected Salmonella will be identified by immunofluorescent staining and flow cytometry.

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 Adam Cunningham. 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 will be done by other members of the research group.

Primary Supervisor:	Francesca Barone
Department:	School of Medicine
Contact:	Dr. Francesca Barone M.D., Ph.D. Wellcome Trust Clinician Scientist School of Immunity and Infection MRC Centre for Immune Regulation College of Medical and Dental Sciences University of Birmingham Edgbaston Birmingham B15 2TT UK Tel +44 (0)1214146480 Fax +44 (0)1214145475 Email : f.barone@bham.ac.uk
Discipline:	Cancer Sciences ☐ Immunology x ☐ Pathology ☐ Anatomy ☐ Metabolic Medicine ☐ Endocrinology ☐ Haematology ☐ Liver & GI Medicine ☐ Infection ☐ Evolutionary basis of clinical medicine ☐

Project Outline

Costimultion blockage in a novel model of tertiary lymphoid neogenesis of the salivary glands.

The interaction between ICOS and its Ligand ICOS-Ligand (ICOS-L) has been identified as a key pathogenic event in the development of autoimmunity. ICOS transgenic mice develop autoimmune features, whereas ICOS-deficient mice notably fail to develop germinal centres (GC) and mount an appropriate T-cell dependent immune response. Increased ICOS expression in humans is believed to be involved in the development of aberrant B cell activation and ectopic lymphoneogenesis. Therefore, inhibiting the ICOS/ICOSL pathway could represent an effective therapy in autoimmunity. OX40 and CD30 regulate memory T cell survival and activation in absence of these molecule lack of appropriate antibody response is onserved. OX40/CD30 knockout mice provide a valuable tool to ascertain the relationship between memory, generation of autoimmunity and formation of chronic lymphocytic infiltrates.

The formaation of ectopic lymphoid-like structures within the target organ of the disease is common feaure of autoimmune conditions. These structures are characterized by follicular dendritic cell positive germinal centres (GCs), T/B cell segregation and formation of high endothelial venules. Our research group have recently developed a novel model of tertiary lymphoid organ formation in murine salivary gland. In this model, murine salivary glands are cannulated via the excretory duct and replication deficient adenovirus constructs carrying luciferase gene infiltrated into the glands. This model has been fully characterized in terms of infiltrate organization and chemokine/cytokine production at different time points. Preliminary experiments have shown at the peak of the inflammatory process (8-10 days post cannulation p.c.) formation of salivary gland inflammatory infiltrates, similar to human pSS, with evidence of GC formation and T/B cell segregation, that spontaneously resolves with virus clearance at around 25 days p.c.

In this project we aim to dissect the role of ICOS/ICOSL and OX40/CD30 interaction

in the formation and organization of the tertiary lymphoid structures in this novel model of salivary gland inflammation.
We will use two integrated approaches:
We will treat cannulated wild type animals (C57/BL6 mice) with anti a novel anti-ICOS compound that has been provided to Dr. Barone by the pharmaceutical company Medimmune. This compound has been already tested for efficacy in blocking ICOS/ICOSL interaction in NOD mouse diabetes.
In order to test the role of OX40/CD30 role in tertiary lymphoneogenesis we will also cannulate OX40/CD30 Ko animals available in this facility.

For the ICOS experiment the compound will be administrated to infected mice at different time points during the inflammatory process (every 2 days from day 6 to day 24) via i.p. injection.
Animals and controls (placebo treated or wild type mice) 4 animals (8 glands) per time point- will be sacrificed every 2 days from day 8 to 24. Submandibular glands snap frozen for analysis and stored at -80. Tissue will be sectioned at the cryostat for immunofluorescence analysis. Thick section (30 microns) will be also collected for mRNA isolation. Immunofluorescence and quantitative real time PCR on whole salivary glands from anti-ICOS treated animals , KO and controls will be performed to evaluate the effects of the lack of costimulation on aggregate organization and cytokine/chemokine production. 1) The number, size and distribution of the inflammatory foci within the glands, their structure in terms of presence and prevalence of T/B cell segregation, formation of follicular dendritic cell networks and high endothelial venules expression of lymphoid chemokines such as CCL21 (T cell attractive) and CXCL13 (B cell attractive) will be analyzed by immunofluorescence. 2) Quantitative real time PCR will be used to assess the expression of cytokines associated with lymphoid organs formation and among those AID, the deaminase regulating the process of antibody affinity maturation. 3) Follicle output in terms of B cell characterization and antibody formation will be also assessed.

How are you planning to ensure adequate supervision?

The student has already familiarized with the research group working as a summer student in 2011. Howevers since she has not been exposed to all the techniques relevant for the project she will receive adequate training for those. The student will also be required to undergo the animal course that will allow her to perform the cannuation on the animals required for the study.
Daily supervision in the lab will be provided by our PhD students (involved in similar projects to that described). All the techniques and the reagents necessary for this study have been already tested and are currently in use in the group.
Dr. Barone will provide technical and scientifical supervion in regular meetings (at least once a week) .

The student role.

The student will be directly involved in all the technical aspects of the project from the cannulation of the animals to the harvest of the organs, storage of the material cutting and process of the samples, data generation and analysis.
Full support and guidance in these processes will be provided by lab members and supervisor.

Please return electronically to Yvonne Palmer, Teaching Support Office, Institute for Cancer Studies or email Y.Palmer@bham.ac.uk

Primary Supervisor:	Prof Chris Buckley with Dr Amy Naylor
Project Title:	The role of CD248 in mesenchymal stem cell differentiation and activity
Department:	Immunity and Infection
Contact:	Email: c.d.buckley@bham.ac.uk **Tel: 0121-414-4383**
Discipline:	Cancer Sciences ☐ Pathology ☐ Metabolic Medicine ☐ Haematology ☐ Infection ☐ Immunology X Anatomy ☐ Endocrinology ☐ Liver & GI Medicine ☐ Evolutionary basis of clinical medicine ☐

Project Outline

We and others have shown that the cell surface receptor CD248 (endosialin) is expressed on pericytes, stromal cells, and cells with MSC properties. (MacFadyen et al., 2005; MacFadyen et al., 2007; Simonavicius et al., 2008). A role for $CD248^{+}$ pericytes in foetal brain angiogenesis has also been defined (Virgintino et al., 2007). CD248 is expressed in a developmentally restricted manner on pericytes and interstitial fibroblasts and a role in tissue remodelling during embryogenesis and immune responses has been suggested (MacFadyen et al., 2007; Rupp et al., 2006; Lax et al., 2007). Our *in vitro* data has shown that CD248 promotes cell proliferation, survival and migration. We now wish to explore the *in vivo* relevance of these observations in models of tissue remodelling and repair using CD248 knockout and transgenic mice and siRNA strategies.

The aim of this project is to elucidate the role of CD248 in MSC differentiation and function. We have preliminary evidence that CD248 modulates MSC differentiation and migration and we wish to increase our understanding of the mechanisms underlying this function.

How are you planning to ensure adequate supervision?

Dr Amy Naylor, a post doctoral Fellow in our group has supervised two successful BMedSci Medical student projects on different aspects of CD248 function in the last year. Most techniques are therefore in place and we have experience of supervising intercalating students and ensuring they have varied and interesting projects.

.

The student role.
Practical Approach Using human/mouse MSC cell lines the student will manipulate CD248 expression using either primary cells derived from CD248-deficient mice or using siRNA to knockdown CD248 in human cells and cell lines. The student will then observe the effect of CD248 over-expression and under-expression on proliferation (^{3}H and CFSE uptake), differentiation (into adipocytes, chondrocytes/osteocytes, myocytes, fibroblasts and pericytes) and migration (chemotactic screens with Dunn Chambers and/or transwell inserts) assays. We will also use PCR and Western blotting to characterise the degree of cell differentiation and to discover which signalling pathways require CD248 (for example PDGF).

Primary Supervisor:	Mark Cobbold and Oliver Goodyear
Project Title:	Developing a Selection Device for Cellular Therapies
Department:	School of Cancer Sciences
Contact:	m.cobbold@bham.ac.uk Tel: 0121 414 6839
Discipline:	Cancer Sciences [ ] Immunology [X] Pathology [ ] Anatomy [ ] Metabolic Medicine [ ] Endocrinology [ ] Haematology [ ] Liver & GI Medicine [ ] **Infection** [ ] Evolutionary basis of clinical medicine [ ]

Project Outline

Patients around the world face terrifying waiting lists for organ transplants. The waiting time for a liver transplant averages 26 months; for a lung, it can be nearly three years. To make matters worse, even if a donor becomes available, there is still a risk that the transplant will be rejected as it is foreign to the patient and rejected by the immune system.

Recent discoveries have increased our understanding of stem cells (the cells which continuously regenerate our tissues) that have led, for the first time, to lab-grown organs being successfully produced for mice. These organs have been able to support life, thus lab-grown organs are increasingly being seen as remedy for long donor waitlists and many chronic diseases. However, before these discoveries can reach patients, there needs to systems in place to culture cells and subsequently purify them before they can be grown or assembled into organs or tissues. Currently there are very few technologies availble that allow the selection of cells at the scale required for human studies.

This project provides an opportunity to develop a new technology to purify cells once they have been grown in a clinically compatible manner. As a starting point we have modified dialysis cartridges, which have an excellent safety record for 'washing' human cells. We can modified 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!) In addition, exposure to very new materials such as monoliths and cryogels, which are similar to sponges will be available. These 'sponges' will be used to further enrich cells so that they will be as close to 100% pure as possible.

The project will culminate with the purification of stem cells from human blood and show that we can purify stem cells and use these to 'grow' blood vessels in the laboratory.

How are you planning to ensure adequate supervision?

Supervision will take place a 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.

Primary Supervisor:	Andrew Filer
Project Title:	Investigating regulation by hypoxia of synovial fibroblast epigenetic modifiers during progression to RA
Department:	School of Immunity & infection/School of Cancer Sciences
Contact:	Email: a.filer@bham.ac.uk
Discipline:	Cancer Sciences ☐ Pathology ☐ Metabolic Medicine ☐ Haematology ☐ Infection ☐ Immunology ■ Anatomy ☐ Endocrinology ☐ Liver & GI Medicine ☐ Evolutionary basis of clinical medicine ☐

Project Outline

The synovial lining layer of the joint in rheumatoid arthritis (RA) is characterised by a hypoxic, pro-inflammatory microenvironment in which stromal synovial fibroblasts play a critical role. They play a direct role in tissue damage both through secretion of multiple matrix metalloproteinases and cathepsins, and promoting differentiation of monocytes into osteoclasts. Attachment to and direct, autonomous invasion of fibronectin-rich matrix such as cartilage is well demonstrated in the SCID mouse model of arthritis, in which cultured RA synovial fibroblasts invade and destroy co-implanted human cartilage. This model demonstrates the remarkably stable and disease specific phenotype of cultured RA synovial fibroblasts, which persists through multiple passages. Evidence is increasing that the phenotypic stability of these fibroblasts is under epigenetic control. Epigenetic modifications to DNA and the histone proteins around which DNA is wrapped in the nucleus are responsible for controlling gene expression, resulting for instance in site specific expression of certain genes, and maintained differentiation of leukocyte subsets. In disease such as cancer and arthritis, these switches may become stably altered resulting in persistent changes in gene expression. However, current investigations of epigenetic modifications in synovial fibroblasts fail to take into account the hypoxic environment that characterises the rheumatoid joint. A critical group of complexes that regulate epigenetic modifications are controlled by the levels of oxygen in the joint. Work in cancer studies (Dr Dan Tennant) is currently characterising the presence and regulation of such complexes in cancer cells. We aim to characterise those same markers in the synovium of patients with normal joints, very early RA when a therapeutic window of opportunity exists, and more established RA. Tissue and fibroblast samples from these patients are already available in the Rheumatology Research Group tissue archive. Tissues from different patient groups will be stained and imaged by confocal microscopy to assess activation of complexes, and prevalant levels of oxidative stress (8-hydroxyguanidine). In *in vitro* assays, fibroblasts will be exposed to hypoxia and pro-inflammatory cytokines, and the presence and activity of complexes measured.

How are you planning to ensure adequate supervision?

The intercalating student will be supervised by Dr Filer, who has supervised such projects successfully before, and co-supervision by Dan Tennant. They will have further additional laboratory supervision in tissue culture and fibroblast biology (Kath Howlett) and training and supervision in confocal microscopy and tissue staining from Fern Barrington and Debbie Hardie.

.

The student role.

1. To understand the background of the study
2. To act as a focus for collaboration between rheumatology and cancer research
3. To learn laboratory techniques:
    a. Tissue culture including hypoxic culture
    b. Tissue processing and staining
    c. Cell biology functional assays: cartilage degradation, migration, invasiveness
    d. Confocal microscopy
    e. Quantitative PCR
    f. Chromatin immunoprecipitation
4. To interrogate tissue samples for markers of epigenetic modifier complexes that are subject to hyxopic control using tissue staining and confocal microscopy techniques
5. To measure expression of such complexes in fibroblasts from different clinical outcome groups
6. To develop an *in vitro* model of the effect of hypoxia on epigenetic regulation in synovial fibroblasts, by exposing fibroblasts to hypoxic conditions ad pro-inflammatory cytokines, and examining the expression of key complexes and characteristic epigenetic marks by PCR and Chromatin immunoprecipitation
7. To have a really good time dipping their toe in science in a forward looking, well funded research group with a great working atmosphere.

Primary Supervisor:	Colin Howard & Jane McKeating
Project Title:	The role of hepatitis B virus proteins in hepatocellular carcinoma.
Department:	School of Immunity and Infection
Contact:	j.a.mckeating@bham.ac.uk
Discipline:	Cancer Sciences ☐ Immunology ☐ Pathology ☐ Anatomy ☐ Metabolic Medicine ☐ Endocrinology ☐ Haematology ☐ Liver & GI Medicine ☐ Infection ☐ Evolutionary basis of clinical medicine ☐

Project Outline

Hepatitis B virus (HBV) is a significant cause of chronic liver disease and hepatocellular carcinoma, with over 250 million infections worldwide. However, the molecular basis of HBV induced liver damage and oncogenesis is poorly defined, in part due to the limited availability of in vitro systems to propagate infectious HBV particles. HBV is known to replicate in hepatocytes, however, the cellular molecules or receptors which define viral tropism for the liver are unknown. Virus engagement of cellular receptors primes signalling events that promote pathogen replication and perturb normal cellular activities1. Thus identification of viral receptors can provide new therapeutic targets for post-exposure anti-viral intervention. We hypothesise that HBV encoded surface antigen (HBsAg) engagement of hepatocyte receptor(s) promotes particle internalization and activates signalling pathways that play a key role in liver injury and tumorigenesis. This project will evaluate the receptor dependency and binding kinetics of HBsAg association with hepatocytes and study the effect(s) on hepatocyte function. This project will address a number of clinically important questions: (1) The role of HBsAg plasma membrane expression as a driver of immune mediated liver damage: and (2) to define the role of HBsAg genetic variability on protein expression and hepatocyte physiology. This study will advance our understanding of HBV-host cell interactions and offers the potential to uncover new avenues for the development of HBV-targeted antivirals

1. Lupberger, J et al. 2011. EGFR and EphA2 are host factors for hepatitis C virus entry and possible targets for antiviral therapy. Nature Medicine 17: 589.
2. Hsu, M et al 2003. Hepatitis C virus glycoproteins mediate pH-dependent cell entry of pseudoparticles. PNAS 11:7271.
3. Harris, H et al 2010. Claudin-1 association with CD81 defines hepatitis C virus entry. J Biol Chem 285: 21092.

How are you planning to ensure adequate supervision?

Professors Howard and McKeating bring together an extensive research experience in hepatitis B and C respectively. This project will provide training in state of the art laboratory techniques in molecular biology, virology, live cell imaging, ex vivo tissue and analysis. The student will learn transferable skills in experimental design, data interpretation, scientific writing, communication and presentation. There will be opportunity to interact with basic scientists (virologists, immunologists) and clinicians practicing in one of the largest liver centres in the UK.

All students in the McKeating laboratory are assigned a post-doctoral scientist to provide training and supervision on the laboratory aspects of the project. The student and Fellow meet with Profs. McKeating and Howard weekly to review data and to plan experimental objectives. In addition to this one-to-one mentoring scheme the student will join a vibrant laboratory of basic and clinical scientists who work in a collaborative manner to study liver disease as evidenced by the labs publication record (**See web links:** HCV Group: School of Immunity and Infection). The HCV research lab holds weekly lab meeting and journal clubs, allowing the student to interact with a wide group of enthusiastic scientists.

The student role.

The student will be trained in cell culture, virus propagation, and liver immunohistochemistry. These techniques provide a generic skill base that is widely applicable to a range of biologically diverse projects.

Primary Supervisor:	Dr Steven Lee (with Prof R. Bicknell)
Project Title:	Targeting the tumour vasculature with genetically modified immune cells.
Department:	School of Cancer Sciences
Contact:	Tel: 0121 414 2803
Discipline:	Cancer Sciences ■ Pathology ☐ Metabolic Medicine ☐ Haematology ☐ Infection ☐ Immunology ■ Anatomy ☐ Endocrinology ☐ Liver & GI Medicine ☐ Evolutionary basis of clinical medicine ☐

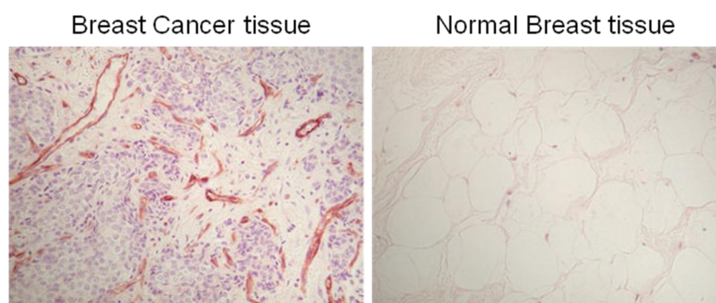
Project Outline

Compared to normal tissue, angiogenesis in tumours is deregulated and/or aberrant, resulting in a structurally and functionally abnormal vasculature. Targeting unique features of the tumour vasculature to compromise blood flow in tumour tissue should therefore provide therapeutic benefit. Anti-angiogenic monoclonal antibodies or small molecules that target these tumour endothelial markers appears to have limited curative potential, possibly because of their cytostatic action and the redundancy of angiogenic pathways. In principle, cytotoxic strategies should be more effective because they could prevent formation of new vessels and destroy existing tumour vasculature.

T lymphocytes are self-replicating effectors that can persist for years and display potent and specific cytotoxic activity. Recent clinical studies infusing cytotoxic T lymphocytes (CTLs) specific for antigens expressed on malignant cells have demonstrated remarkable efficacy in treating metastatic melanoma[1]. However, extending this therapy to other cancers is limited by a lack of appropriate tumour antigens. Targeting T cells to tumour endothelial markers offers an alternative approach that in animal models has been shown to inhibit tumour growth and prolong host survival[2,3].

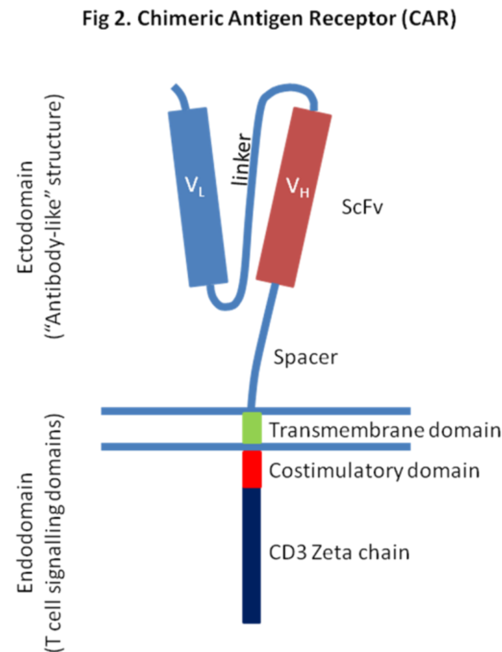
Our recent work has identified a novel tumour endothelial marker, CLEC14A[4]. This C-type lectin is highly expressed on the surface of endothelial cells lining the vasculature of many common human cancers (including breast, prostate, bladder and ovarian carcinomas) but in the vasculature of healthy tissue expression is low or undetectable (Fig. 1).

Fig. 1 CLEC14A is strongly expressed on blood vessels within breast cancer tissue but not in normal breast tissue.



It is now possible to engineer T cells with a defined specificity by transducing genes encoding so-called "chimeric antigen receptors" (CARs). CARs combine the specificity of antibodies with the cytotoxic and immunomodulatory functions of T cells and function in an MHC-unrestricted manner (reviewed[5]). Typically, CARs consist of a single chain variable fragment (scFv) of a specific antibody linked to intracellular T cell signalling domains (Fig. 2).

Fig 2. Chimeric Antigen Receptor (CAR)



The anti-tumour effects of CAR-expressing T cells have been demonstrated in pre-clinical models[6] and more recently in clinical trials[7,8]. Using a panel of novel monoclonal antibodies specific for human CLEC14A, we plan to generate CAR constructs that will target T cells to recognise and destroy the tumour vasculature.

Aims: Generation and characterisation of CLEC14A-specific CARs to assess their therapeutic potential for cancer.

The project will focus on the following three areas:

1. Using PCR, single chain variable fragment (scFv) genes will be isolated from the hybridomas that express CLEC14A-specific antibodies. These will be cloned into appropriate expression vectors for sequencing and testing for CLEC14A-specific binding.

2. The scFv gene will then be cloned into an existing retroviral expression plasmid designed to generate the CAR construct. Human T cells will be transduced in vitro using this retrovirus to stably express the CAR on the cell surface. Expression of the CAR will be explored using flow cytometry and the specificity and function of CAR-expressing T cells will be tested using several assays of T cell function exploring their ability to proliferate and release cytokines in response to CLEC14A and their ability to kill CLEC14A-expressing target cells.

3. Further studies will explore the optimal CAR design, incorporating different costimulatory domains and spacer regions to maximise T cell responses in

vitro and thereby the therapeutic potential of such T cells.

This project is based on a joint study between two labs in the medical school, combining the expertise of Dr Steve Lee (T cell therapy including engineering T cells to express cloned antigen receptors) and Prof Roy Bicknell (angiogenesis).

References

1. Rosenberg, SA et al. Clin Cancer Res, (2011).
2. Chinnasamy, D et al. J Clin Invest **120**, 3953, (2010).
3. Niederman, TM et al. Proc Natl Acad Sci U S A **99**, 7009, (2002).
4. Mura, M et al. Oncogene, in press, (2011).
5. Sadelain, M et al. Curr Opin Immunol **21**, 215, (2009).
6. Brentjens, RJ et al. Nat Med **9**, 279, (2003).
7. Porter, DL et al. N Engl J Med **365**, 725, (2011).
8. Pule, MA et al. Nat.Med. **14**, 1264, (2008).

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 Bicknell labs. 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 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.

Primary Supervisor:	Jane A McKeating & Garrick K Wilson
Project Title:	A role for hepatitis c virus, chemokine receptors in hepatocellular carcinoma.
Department:	School of Immunity and Infection
Contact:	j.a.mckeating@bham.ac.uk
Discipline:	Cancer Sciences ☐ Pathology ☐ Metabolic Medicine ☐ Haematology ☐ Infection ☐ Immunology ☐ Anatomy ☐ Endocrinology ☐ Liver & GI Medicine ☐ Evolutionary basis of clinical medicine ☐

Project Outline

Hepatitis C virus (HCV) infection is associated with the development of hepatocellular carcinoma (HCC); an aggressive tumour of the liver with limited treatment options and poor prognosis, highlighting the need to increase our understanding of the role HCV infection plays in carcinogenesis. HCC is an aggressive and rapidly fatal malignancy representing the fifth most common cancer worldwide. Although surgical techniques and perioperative care have improved in recent years, the long-term prognosis is poor due to its high metastatic capacity. Organ-specific metastasis is governed, in part, by interactions between chemokine receptors on cancer cells and matching chemokines in target organs. Chemokine receptors have been reported to play a role in HCC carcinogenesis[1], however, the exact mechanisms are unknown.

We recently reported that HCV infection stabilizes hypoxia inducible factor 1α, a regulator of the transcriptional response to oxygen deprivation and genes involved in angiogenesis, cell invasion and metastasis[2]. The independent observation that hypoxia or low oxygen levels regulate CXCR-4[3] in breast cancers prompted us to study the effect(s) of HCV infection on CXCR4 expression. Current experiments show that HCV infection promotes CXCR4 expression and localization at the plasma membrane, suggesting a direct role for the virus in promoting hepatoma migration and metastasis. This project will address the functional consequences of CXCR4 expression in HCV infected cells and will assess the activity of CXCR4 antagonist AMD310 to specifically target HCV infected hepatoma cells. This project will increase our understanding of HCC pathology and ability to more efficiently target metastatic cancer cells.

1.Roberts LR. 2005. Chemokines as attractive targets in liver carcinogenesis. Am J Gastroenterol 100:499.
2.Wilson, G.K., Brimacombe, C.L., Rowe, I.A et al. 2011. A dual role for hypoxia inducible factor-1α in the hepatitis C virus lifecycle and hepatoma migration. J Hepatol, in press.
3.Staller, eSulitokova, J., Lisztwan, J., et a. 2003. Chemokine receptor CXCR4 downregulated by von Hipel-Lindau tumour suppressor pVHL. Nature 425: 307.

How are you planning to ensure adequate supervision?
All students in the McKeating laboratory are assigned a post-doctoral scientist to provide training and supervision on the laboratory aspects of the project. The student and fellow meet with Prof. McKeating weekly to review data and to plan experimental objectives. In addition to this one-to-one mentoring scheme the student will join a vibrant laboratory of basic and clinical scientists who work in a collaborative manner to study liver disease as evidenced by the labs publication record (**See web links:** HCV Group: School of Immunity and Infection). The HCV research lab holds weekly lab meeting and journal clubs, allowing the student to interact with a wide group of enthusiastic scientists.
The student role.
The student will be trained in cell culture, cell migration assays, virus propagation, confocal imaging to monitor CXCR4 trafficking liver immunohistochemistry. The data will form part of a larger study that will be submitted for publication to a high impact journal. These techniques provide a generic skill base that is widely applicable to a range of biologically diverse projects.

Primary Supervisor:	Dr Matthew Morgan Professor Lorraine Harper Dr Julie Williams
Project Title:	The role of the inflammasome in renal inflammation
Department:	Centre for Translational Inflammation Research
Discipline:	Cancer Sciences ☐ Immunology X ☐ Pathology ☐ Anatomy ☐ Metabolic Medicine ☐ Endocrinology ☐ Haematology ☐ Liver & GI Medicine ☐ Infection ☐ Evolutionary basis of clinical medicine ☐

Project Outline

Anti-neutrophil cytoplasm antibody (ANCA) associated vasculitis is a life-threatening autoimmune disease. ANCA are auto-antibodies against the neutrophil enzymes myeloperoxidase or proteinase 3 which activate circulating cytokine primed neutrophils leading to adhesion to endothelium, degranulation, cytokine release and superoxide production which causes tissue damage.

We have previously shown that IL-18 is produced by podocytes in the glomerulus and primes neutrophils for activation by ANCA. Production of IL-1b and IL-18 requires 2 separate signals; NF-κB activation induces the synthesis of pro-IL-1b and pro-IL-18 followed by a second signal that triggers proteolytic pro-IL-1b and pro-IL-18 processing, mainly by caspase-1, to produce the bioactive cytokines. Pro-caspase-1 activation requires the recruitment and oligomerisation of the enzyme within a molecular platform known as the inflammasome.

NLRP3 is the best understood inflammasome detecting several pathogen associated and damage associated molecular patterns (DAMPs). ANCA activated neutrophils undergo accelerated apoptosis and necrosis as well as respiratory burst releasing cell contents and superoxide that may activate NLRP3. However serine proteases, including proteinase 3 (PR3), released during ANCA stimulated degranulation have also been shown to process pro-IL-1b and pro-IL-18 bypassing caspase-1 and the inflammasome.

If neutrophil activation in the glomerulus leads to production of IL-18 which then drives further neutrophil activation then there is a positive feedback loop promoting ongoing inflammation and damage which may be amenable to therapeutic blockade.

You will investigate whether bacterial lipopolysaccharide acting via TLR4 activates podocyte NFkB leading to increased cytokine synthesis. You will also investigate whether proteinase 3 released from neutrophils can process the pro-cytokines to their active forms.

The expression of TLR-4 on cultured conditionally immortalised podocytes will be confirmed by flow cytometry. LPS activation of NFkB and the increased synthesis of IL-1b and IL-18 will be confirmed by Western blotting. Following the addition of recombinant proteinase 3 to LPS activated podocytes you will investigate whether PR3 is able to process pro-IL-1b and pro-IL-18 to their active forms. You will investigate whether the presence of degranulated or necrotic neutrophils leads to processing of IL-18 either by activation of the inflammasome or by proteinase 3 from the neutrophil bypassing the action of caspase-1.

Cultured conditionally immortalised podocytes will be incubated with degranulated or

apoptotic neutrophils (in response to ANCA and other stimuli). The release of processed IL-18 and IL-1b from podocytes will be determined by western blotting. Repeating the experiment in the presence of protease inhibitors or antioxidants will determine whether proteinase 3 or superoxide are important in the activation of the inflammasome and processing of pro-IL-18 and pro-IL-1b.

How are you planning to ensure adequate supervision?

The student will be taught the laboratory techniques necessary for the project. There will be weekly (minimum) meetings with the supervisors to monitor progress. All three supervisors are based within the laboratory area with an open access policy for students to allow any problems/questions to be dealt with immediately.

The student role.

You will learn cell culture techniques to enable you to grow conditionally immortalised podocytes under various experimental conditions.
You will learn how to do Western blotting to determine the podocyte expression of various receptors and enzymes as well as pro- and bioactive cytokines produced in response to experimental conditions.
You will learn how to isolate neutrophils from blood donated from healthy volunteers.
You will learn how to purify immunoglobulin G from healthy donor serum and ANCA containing patient serum.
You will learn how to cytokine prime isolated neutrophils and activate them with ANCA containing IgG or normal IgG and use these to investigate the effect on cytokine production in podocytes.
You will learn the relevant statistical methods to enable you to determine the significance of your experimental findings.

Primary Supervisor:	Dr Neil Morgan
Project Title:	Molecular Genetic Investigation of autosomal recessively inherited immune disorders
Department:	School of Clinical and Experimental Medicine
Contact:	Email: n.v.morgan@bham.ac.uk Tel: 0121 415 8678
Discipline:	Cancer Sciences ☐ Pathology ☐ Metabolic Medicine ☐ Haematology ☐ Infection ☐ Immunology ■ Anatomy ☐ Endocrinology ☐ Liver & GI Medicine ☐ Evolutionary basis of clinical medicine ☐

Project Outline

Outline of the Project

The identification for the molecular basis of rare genetic disorders can provide insights into fundamental biological pathways and the pathogenesis of common diseases. Advances in genomic technologies have greatly facilitated the identification of human disease genes. Consanguineous families with autosomal recessive disorders provide a very powerful resource for gene mapping and we have found that searching for regions of homozygosity in affected individuals from consanguineous families (autozygosity mapping) represents a highly efficient and effective approach for generating disease gene localisation even in the presence of locus heterogeneity. Thus using this approach we have identified novel genes for a number of immune disorders [1-3]. Recently, we have found that a combination of autozygosity mapping and whole-exome sequencing by second generation sequencing can greatly facilitate the identification of recessive disease genes [2].
In order to identify novel genes for autosomal recessively inherited immune disorders, we have ascertained consanguineous families from the West Midlands through the Regional clinical immunology service at Heartlands Hospital and established a collaboration with the national immunodeficiency service in Newcastle. This has identified a cohort of families suitable for gene identification studies which will be used in this project, by combining the highly successful techniques of autozygosity mapping and whole exome sequencing.

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, Rahman F, Barge D, Ciupek A, Straatman-Iwanowska A, Pasha S, Guckian M, Anderson G, Huissoon A, Cant A, Tate WP, Hambleton S, Maher ER (2011) Mutation in the TCRα subunit constant gene (*TRAC*) leads to a human immunodeficiency disorder characterized by a lack of $TCR\alpha\beta^{+}$ T cells. J Clin Invest 121(2):695-702.
[2] Bolze A, Byun M, McDonald D, Morgan NV, Abhyankar A, Premkumar L, Puel A,

Bacon CM, Rieux-Laucat F, Pang K, Britland A, Abel L, Cant A, Maher ER, Riedl SJ, Hambleton S, Casanova JL (2010) Whole-exome-sequencing-based discovery of human FADD deficiency. Am J Hum Genet 87(6):873-81.
[3] Morgan NV, Morris MR, Cangul H, Gleeson D, Straatman-Iwanowska A, Davies N, Keenan S, Pasha S, Rahman F, Gentle D,Vreeswijk MPG, Devilee P, Knowles MA, Ceylaner S, Trembath RC, Dalence C, Kismet E, Koseoglu V, Rossbach H-C, Gissen P, Tannanhill D, Maher ER (2010). Mutations in SLC29A3, encoding an equilibrative nucleoside transporter ENT3, cause a familial Histiocytosis syndrome (Faisalabad histiocytosis) and Familial Rosai-Dorfman disease. PLoS Genetics 6(2):e1000833.

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 phenotype of the immune disorders.
The immediate focus of the planned project will be the identification of new causative genes using the powerful combination of autozygosity mapping (Affymetrix v6.0 SNP array and microsatellite marker analysis) with large-scale exome sequencing (using the Illumina Genome Analyzer IIx).
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 immune disorders leading to a publication.

Primary Supervisor:	Dr Katie Hardy and Professor Peter Hawkey
Project Title:	Identification and amplification of known and novel repetitive DNA sequences for the control of Staphylococcus aureus cross infection
Department:	Infection and Immunity
Contact:	Email: Katie.hardy@heartofengland.nhs.uk Tel: 0121 424 2465
Discipline:	Cancer Sciences ☐ Pathology ☐ Metabolic Medicine ☐ Haematology ☐ Infection ■ Immunology ☐ Anatomy ☐ Endocrinology ☐ Liver & GI Medicine ☐ Evolutionary basis of clinical medicine ☐

Project Outline

Methicillin resistant *Staphylococcus aureus* is a healthcare associated infection that can cause a considerable degree of morbidity and mortality. A range of infection control measures have been introduced in an effort to control the spread of MRSA, and although rates have reduced none have been able to eliminate MRSA from the hospitals. A greater understanding of the genetic relatedness and spread of clonal strains is necessary in order to be able to control MRSA in both endemic and outbreak situations. Numerous methods are available for typing MRSA all of which have limitations. Variable number tandem repeats (VNTRs) have been increasingly used as markers for strain typing of various bacteria. Determination of the numbers of repeats at each locus produces a digital profile providing a highly portable typing method allowing comparison between laboratories. Our group described the presence of VNTRs in staphylococci, termed staphylococcal interspersed repeat units (SIRUs) and their ability to discriminate between the main epidemic MRSA (EMRSA) within the UK.

At present the VNTR typing scheme applied routinely to investigate clusters of MRSA within hospitals uses seven loci. The aim of this project is to identify and investigate novel repetitive DNA sequences that may provide greater discriminatory ability and aid in the investigation of both the evolution and detailed epidemiology of MRSA.

The project will give a wide range of training in basic microbiology skills, molecular biology and bioinformatics.

How are you planning to ensure adequate supervision?

Prof Hawkey and Dr Hardy will meet fortnightly with the student to agree specific research tasks, daily supervision will be by Dr Hardy and other members of the Hawkey team.

The student role.
The student will be required to complete literature searches and in conjunction with the supervisors plan the programme of research. The work will be undertaken within the laboratories both at the University of Birmingham and in the Health Protection Laboratories at the Heart of England NHS Foundation Trust. The student will be required to present their work at the hawkey team research meetings.

Primary Supervisor:	Dr Mark A Webber
Project Title:	New strategies to fight healthcare infection
Department:	School of Cancer Sciences
Contact:	Email: m.a.webber@bham.ac.uk Tel: 0121 414 2859
Discipline:	Cancer Sciences ☐ Immunology ☐ Pathology ☐ Anatomy ☐ Metabolic Medicine ☐ Endocrinology ☐ Haematology ☐ Liver & GI Medicine ☐ Infection x☐ Evolutionary basis of clinical medicine ☐

Project Outline

The rise and spread of antibiotic resistance is one of the great challenges to global health of the 21st century and was the topic of the WHOs 'World health day' in 2011. The emergence of pathogens resistant to all therapeutically useful antibiotics is compromising not only the ability to treat life threatening infections but also presenting a challenge for various routine surgeries and other procedures where antibiotic prophylaxis is critical. There is a lack of novel antibiotic development with no new drugs approaching clinical utility; consequently the prevention of infection is of paramount importance. One of the mechanisms by which bacteria can resist the action of antibiotics and biocides is by production of multidrug resistance (MDR) efflux pumps that export antibiotics and other toxic molecules. These transporters are found in all cells and have been a research focus for our laboratory in the last ten years. One key finding we have made is an association between MDR efflux pumps and the ability to form a biofilm. Biofilms are bacterial communities attached to a surface which are extremely drug resistant and provide a reservoir for persistent infection in the hospital environment. We have shown that genetic inactivation of various MDR pumps results in a loss of the ability to form a biofilm and that various chemical efflux inhibitors can exert the same effect against *Salmonella*, a major food borne pathogen.

In this project we will evaluate the effectiveness of a range of chemical efflux inhibitors in the inhibition of biofilm formation by various multi-resistant pathogens (*E. coli*, *S. aureus*, *K. pneumoniae*) on surfaces found in the ICU. Thereby this project will identify whether this novel route of intervention is feasible and worthy of further development as an anti-infective strategy.

The project will involve a range of microbiological and molecular techniques all of which are up and running in the host laboratory.

How are you planning to ensure adequate supervision?

Dr Webber will meet the student weekly for a formal supervision meeting, in addition, daily supervision will be by Miss Stephanie Baugh and Dr Webber as well as by other members of the Webber laboratory as required.

The student role.

The student will be responsible for performing and analysing experiments under the direction of Dr Webber and Miss Baugh 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

Primary Supervisor:	Dr Mark A Webber
Project Title:	Do bacteria modify gene expression in response to antibiotic stress in a generic, protective way?
Department:	School of Cancer Sciences
Contact:	Email: m.a.webber@bham.ac.uk Tel: 0121 414 2859
Discipline:	Cancer Sciences ☐ Immunology ☐ Pathology ☐ Anatomy ☐ Metabolic Medicine ☐ Endocrinology ☐ Haematology ☐ Liver & GI Medicine ☐ Infection x☐ Evolutionary basis of clinical medicine ☐

Project Outline

The rise and spread of antibiotic resistance is one of the great challenges to global health of the 21st century and was the topic of the WHOs 'World health day' in 2011. The emergence of pathogens resistant to all therapeutically useful antibiotics is compromising not only the ability to treat life threatening infections but also presenting a challenge for various routine surgeries and other procedures where antibiotic prophylaxis is critical.

Recently it has been suggested that all bactericidal antibiotics will interact with a specific cellular target but in addition induce a signalling cascade in the bacterial cell which results in production of reactive oxygen species, cellular damage and contributes to cell death. One important class of bactericidal antibiotics are the quinolones which specifically target DNA gyrase at the GyrA subunit. Gyrase is involved in maintaining the global level of DNA supercoiling i.e. how tightly coiled the DNA is, mutations within gyrase alter the global level of DNA supercoiling. This can, in turn influence numerous other cellular processes including transcription; the primary level at which bacterial gene expression is regulated. We have observed the frequent selection of mutants with changes within GyrA after exposure to a range of bactericidal stresses (non quinolone drugs) which do not directly target gyrase. We hypothesise that mutants with a mutant gyrase have altered expression of genes which are generically protective against antimicrobial treatment and this is why they are being selected in an evolutionary manner. In support of this idea analysis of all the genes expressed in a GyrA mutant revealed increased expression of various known stress response pathways.

This project aims to explore this hypothesis by determining whether GyrA mutants survive exposure to non quinolone antibiotics better than parent strains and determining the basis for this increased survival. The project will inform appropriate use of antibiotics and identify conditions which might select for development of resistance which are currently unidentified. The project will involve a range of microbiological and molecular techniques all of which are up and running in the host laboratory.

How are you planning to ensure adequate supervision?

Dr Webber will meet the student weekly for a formal supervision meeting, in addition, daily supervision will be by Dr Rebekah Whitehead and Dr Webber as well as by other members of the Webber laboratory as required..

The student role.
The student will be responsible for performing and analysing experiments under the direction of Dr Webber and Dr Whitehead 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

Primary Supervisor:	Professor LJV Piddock
Project Title:	Multi-drug resistance in *Acinetobacter spp*
Department:	School of Cancer Sciences
Contact:	Email: l.j.v.piddock@bham.ac.uk Tel: 0121 414 6955
Discipline:	Cancer Sciences [ ] Pathology [ ] Metabolic Medicine [ ] Haematology [ ] Infection [x] Immunology [ ] Anatomy [ ] Endocrinology [ ] Liver & GI Medicine [ ] Evolutionary basis of clinical medicine [ ]

Project Outline

Acinetobacter spp. are commonly implicated in nosocomial infections and cause a significant number of opportunistic infections such as pneumonia, urinary tract and skin and soft tissue infections, particularly in intensive care units and in military casualties returning from combat zones such as Iraq and Afghanistan. *Acinetobacter* is intrinsically resistant to a number of antibiotics and has the ability to rapidly acquire resistance determinants, making it possible for a strain to become pan-resistant and therefore untreatable. Multi-drug resistant (MDR) *Acinetobacter* infections result in increased morbidity rate, mortality rate and health care costs. MDR *Acinetobacter spp.* have caused serious problems in Singapore for over a decade and are now being recognized as a major problem in the UK. Carbapenems are often the only treatment option against MDR strains, however resistance to this antibiotic class has emerged in the clinical setting both in developing and developed countries. In 2006, approximately 50% of all *Acinetobacter spp.* isolated in Singapore public-sector hospitals were resistant to carbapenems and in a recent Health Protection Agency report, imipenem resistance in *Acinetobacter baumannii* bacteraemia isolates had risen from 21% in 2006 to 27% in 2010 in the UK (http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/Acinetobacter/Surveillance/) leaving last line antibiotics such as tigecycline and colistin and novel combinations of antibiotics as the only therapeutic options. A common cause of MDR is innate and over-production of efflux pumps that export antibiotics from the bacterial cell.

In this project we will explore the role of multi-drug efflux pumps in conferring all or some of the MDR in *Acinetobacter* strains and isolates from patients from Singapore and Birmingham (including military patients). Experiments will be carried out that will inform therapeutic strategies to minimise resistance during treatment of MDR *Acinetobacter*. These include:

- Measuring the effect of different antibiotics, biocides (antiseptics and disinfectants) and environmental conditions (e.g. temperature) upon the amount of efflux pump produced. This assay will be carried out in a 96 well automated format.
- Experiments to show which combination of agents/conditions prevent production of efflux. Recently, we and others have shown that some licensed drugs that are not antibiotics can affect the production of efflux pumps. Synergistic and antagonistic combinations of antibiotics and non-antibiotics will be identified in a chequerboard MIC test.

How are you planning to ensure adequate supervision?
Prof. Piddock will have regular one to one meetings at least once per week with the student. Daily supervision will be by Miss Grace Richmond, MRC funded technician and Miss Laura Evans (HPA funded PhD student) who both work on Acinetobacter, in the Piddock team. Additional supervision is also available from Dr Vito Ricci and Dr Jessica Blair, also in the Piddock team.
The student role.
The student will be responsible for performing and analysing experiments under the direction of Prof Piddock and Miss Richmond 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

Primary Supervisor:	Dagmar Scheel-Toellner / Karim Raza
Project Title:	How do B cells contribute to joint damage in patients with rheumatoid arthritis?
Department:	School of Cancer Sciences
Contact:	Email: d.scheel@bham.ac.uk Tel: 0121 415 8690
Discipline:	Cancer Sciences [ ] Immunology [X] Pathology [ ] Anatomy [ ] Metabolic Medicine [ ] Endocrinology [ ] Haematology [ ] Liver & GI Medicine [ ] Infection [ ] Evolutionary basis of clinical medicine [ ]

Project Outline

Background: RANKL, a cytokine belonging to the TNF family, drives bone erosion by inducing proliferation, differentiation and activation of osteoclasts (1). Therapeutic antibody blockade of RANKL is effective at suppressing joint erosion in patients with rheumatoid arthritis (RA). In patients with RA, RANKL has been widely reported to be produced by activated T cells and fibroblasts. In a non-biased study of cytokine mRNA expression in ex vivo sorted inflammatory cells, we observed expression of RANKL in synovial fluid (SF) B cells at the mRNA and the protein levels (2). When we compared levels of RANKL mRNA expression in SF and PB CD4+ and CD8+ T cells, B cells, macrophages and neutrophils, levels of expression were highest in the SF B cell population. These findings suggested a role for B cells in joint destruction by production of RANKL. Our observation that B cells at the site of inflammation produce this cytokine also provides a plausible explanation for the inhibition of joint erosion by B cell targeting therapies. We tested whether treatment with rituximab, an antibody based therapeutic that leads to removal of B cells, affects RANKL expression in the synovium. Following rituximab treatment, RANKL expression is significantly reduced in the synovium (3), suggesting that removal of B cells leads to a decrease in local RANKL levels. Currently we are using a 9 colour panel of antibodies characteristic for B cell subsets to characterise the RANKL producing B cells in RA. So far, data suggest that while the RANKL expressing B cells are enriched in the CD27low/IgD- subset, several B cell subsets express RANKL in the synovium. Therefore, it appears that synovial B cells expression of RANKL reflects stimulation by a yet undefined mechanism rather than differentiation of a defined B cells subset. **We therefore hypothesise that factors present in the synovial fluid and tissue stimulate local production of RANKL by B cells, contributing to joint destruction.**

Aims: In this project we plan to investigate the signals that induce RANKL expression by B cells. We will stimulate sorted B cells *in vitro* to assess the effect of anti-CD40 or anti-IgG and-IgM antibodies alone or in combination with B cell stimulating cytokines (BLyS, APRIL, IL-4, IL-5, IL-6 and IL-10) and investigate RANKL production by flowcytometry, ELISA and on the mRNA level by quantitative PCR. We will apply these stimuli to B cells isolated from peripheral blood of healthy controls and RA patients as well as from synovial fluid of RA patients. To investigate the effect of the combined local cytokine environment in the RA joint we will test the effect of adding RA synovial fluid to B cells isolated from peripheral blood on RANKL expression and release.

1. Kong, Y. Y., Kishihara, K., Yoshida, H., Mak, T. W., & Nomoto, K. (1995) *J*

Immunol **154,** 5725-5735.
2. Yeo, L., Toellner, K. M., Salmon, M., Filer, A., Buckley, C. D., **Raza, K., & Scheel-Toellner, D.** 2011 *Ann Rheum Dis* **70,** 2022-2028.
3. Boumans, M. J., Thurlings, R. M., Yeo, L., **Scheel-Toellner, D.**, Vos, K., Gerlag, D. M., & Tak, P. P. 2011 *Ann Rheum Dis* **71,** 108-113.

How are you planning to ensure adequate supervision?

Dr Scheel-Toellner is a non-clinical scientist with a strong interest in the role of the immune system in the pathogenesis of chronic inflammatory diseases. Her group (currently 1 post-doc, 3 PhD students, a technician and a visiting PhD student from a collaborating Lab in Spain) specialises in the investigation of disease mechanisms in *ex-vivo* samples from patients with RA and SLE. They use a range of cutting edge technologies to dissect the contribution of several types of immune cells.
She has successfully coached four PhD students through their respective PhD projects and maintains an open door policy for her group to enable ad-hoc discussions and troubleshooting. In addition, there are scheduled group and individual meetings. The longstanding and successful collaboration with clinical rheumatologists Drs Karim Raza and Andrew Filer and the wider context of the Rheumatology Research Group are an ideal environment for training in basic and translational research of chronic inflammatory diseases.

The student role.

We can offer a world class, highly supportive research environment. However, we also have high expectations of the contribution of the students. We expect them to actively engage with their project, read the relevant literature and keep clear records. While we will undertake to carefully train the students in the techniques used; they will then have to take responsibility for their own work and make it "their own project". Working with clinical samples often requires flexibility and does sometimes not fall into a 9-5 working day. Importantly, we expect them to join in the discussions within the Rheumatology Research Group and interact positively with the rest of the team.
We also expect the students to consider the legal issues of working with patient material in the context of the Human Tissue Act and follow health and safety instructions to not put themselves or their colleagues at risk. In the context of health and safety issues associated with clinical samples, the students should be vaccinated against Hepatitis B.

Primary Supervisor:	Dr Stephen Young
Project Title:	Could Lyp phosphatase variants affect phagocyte function and contribute to disease progression in rheumatoid arthritis and vasculitis?
Department:	School of Immunity & Infection
Contact:	Email: s.p.young@bham.ac.uk Tel: 0121 414 5475
Discipline:	Cancer Sciences ☐ Pathology ☐ Metabolic Medicine ☐ Haematology ☐ Infection ☐ Immunology ■ Anatomy ☐ Endocrinology ☐ Liver & GI Medicine ☐ Evolutionary basis of clinical medicine ☐

Project Outline

A single nucleotide polymorphism in the PTPN22 gene is strongly associated with a number of autoimmune diseases including rheumatoid arthritis and primary vasculitis [1]. The variant gene gives rise to a form of the Lyp phosphatase that fails to interact with the kinase csk [2]. This disrupts the regulation of the key TCR signalling kinase Lck and so may alter the process of negative selection of autoreactive T cells in the thymus[1]. This variant also has effects in mature lymphocytes in the form of poor or depressed signalling through the TCR and BCR [3]. However, the Lyp protein is expressed at the highest levels in phagocytes and at only modest levels in lymphocytes [4]. The synovial membrane in rheumatoid arthritis, like most inflammatory sites, contains large numbers of macrophages and neutrophils, which contribute to joint damage. It is possible then that the dysfunction of the variant Lyp in phagocytes makes a substantial contribution to tissue damage in these diseases by promoting dysfunction of the phagocytes. In primary vasculitis autoantibodies against neutrophils activate these cells leading to widespread vascular and organ (renal) damage. In patients with the variant PTPN22, which is expressed at high levels in these cells, altered neutrophil function could contribute to their hyperactivity in this disease and promote damage.

However, the function of Lyp in macrophages and neutrophils has not been studied .
The student will test the hypothesis that PTPN22 regulates phagocyte activation and adhesion.
We will knock down Lyp expression in HL60, THP1 and U937 cell lines and study their responses to FcR and TLR ligation.

We will transfect shRNA to knockdown Lyp expression. By using a viral plasmid construct (Qiagen SABiosciences) permanent knockdown should be achievable. Lyp phosphatase activity will be assessed using an assay we developed for CD45 [5]. Levels of PTPN22 protein will be monitored using western blotting and mRNA using RT-PCR. Calcium signalling in response to TLR and FcR ligation will be assessed along with production of superoxide (through cytochrome C reduction). Adhesion of the depleted HL60 to substrates in a static adhesion assay will also be assessed. Whole cell phosphotyrosine immunoblots, before and after activation, will reveal if substantial changes in phosphorylation are observed and possibly identify specific hyperphosphorylated targets of Lyp in phagocytes. Neutrophils will be isolated from patients with the variant allele and their activation, adhesion and function studied.

1. Vang T, et al. Protein tyrosine phosphatase PTPN22 in human autoimmunity. Autoimmunity. 2007;40(6):453-61.
2. Bottini N, et al. A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. Nat Genet. 2004;36(4):337-8.

3. Rieck M, et al. Genetic variation in PTPN22 corresponds to altered function of T and B lymphocytes. J Immunol. 2007;179(7):4704-10.
4. Chien WW, et al. Characterization of a myeloid tyrosine phosphatase, Lyp, and its role in the Bcr-Abl signal transduction pathway. J Biol Chem. 2003;278(30):27413-20.
5. Rider DA, Young SP. Measuring the specific activity of the CD45 protein tyrosine phosphatase. J Immunol Methods. 2003;277(1-2):125-32.

How are you planning to ensure adequate supervision?

The student will be part of the Rheumatology Research Group in the new Centre for Translational Research in Inflammation in the QE Hospital where we have a number of PhD students, technicians and Post-docs working on aspects of immune function in RA and so there will always be support available. Dr Young is actively engaged in laboratory research and so will provide the initial training for the student and will be present throughout the year for close supervision.

.

The student role.

The student will gain experience in a wide variety of techniques starting with the expression of plasmids in E. coli, their isolation, and transfection into mammalian cells. Western blotting and real-time PCR will be used to determine the efficacy of the knockdown process. Tissue culture of a range of mammalian phagocyte lines will be the responsibility of the student, who will also analyse calcium signalling and protein tyrosine phosphorylation in these cells using fluorimetry and western blotting. Isolation of neutrophils from blood of patients with vasculitis and rheumatoid arthritis will also be undertaken for assessment of the effect of variant PTPN 22 on the function of these cells. The student will be expected to attend the weekly rheumatology research group meetings and also to present at these.

Primary Supervisor:	Constanze Bonifer
Project Title:	Aberrant activation of repeat elements in classical Hodgkin's Lymphoma and Acute Myeloid Leukaemia and their role in cancer pathogenesis
Department:	School of Cancer Sciences
Contact:	Email: c.bonifer@bham.ac.uk Tel: 0121 414 8881
Discipline:	Cancer Sciences ☐ Pathology ☐ Metabolic Medicine ☐ Haematology X Infection ☐ Immunology ☐ Anatomy ☐ Endocrinology ☐ Liver & GI Medicine ☐ Evolutionary basis of clinical medicine ☐

Project Outline

Background and purpose:

Retroviruses such as HIV infiltrate the human genome by integrating their genetic material into the genome of infected cells and this can lead to aberrant gene expression that is driven by the Long Terminal Repeats (LTRs) at the end of the viral genome. In an evolutionary arms race mammals developed the ability to recognize and silence transcription from these intruding genetic elements. This is mediated by epigenetic control such as the specific methylation of retroviral DNA. During evolution, the remnants of this conflict have accumulated in our DNA and a significant fraction of the human genome (approx 8%) consists now of retroviral and retrotransposon sequences. Although it has known for some time that such repeat elements are aberrantly activted in cancer, the impact of these sequences on host gene expression and their contribution to human disease are only beginning to be understood.

My lab showed that certain types of human lymphoma (Hodgkin Lymphoma, HL) are characterised by the aberrant activation of a specific class of LTRs, and we demonstrated that one of these LTRs activated an oncogene (*CSF1R*) that is essential for HL cell growth (Lamprecht et al., Nature Medicine, 2010, for more explanation hear my podcast on http://www.birmingham.ac.uk/staff/profiles/cancer/bonifer-constanze.aspx). Our study demonstrating that this oncogene is expressed from an aberrantly activated LTR in HL was one of the first cases of a gene involved in tumour pathogenesis that is expressed from such an element. Moreover, using generic primers for this class of LTRs we also showed that their activation in HL is wide-spread and each cell line expressed a different pattern of LTR-driven genes. However, currently many questions are still open with respect to the full impact of the activation of these normally silent DNA elements with respect to tumour development and maintenance. For example, it is highly likely that the extensive activation of repeat elements is one of the reasons for the extreme genomic instability of HL cells. We also do not know whether LTR activation is HL-specific or also found in other cancers.

The project described here will build on these observations and will use experimental and computational methods to answer answer the following questions:

1. What is the full complement of activated LTRs in HL cells?

In our previous study we used LTR-class specific mRNA primers to clone a small selection of individual different elements and sequenced them individually. We now wish to obtain a more global picture of which LTRs are activated, which genes they regulate and whether these genes potentially play a role in tumour pathogenesis. The student therefore will prepare cDNA libraries from all mRNAs originating within LTRs. These fragments will first be analyzed by

classical sequencing, but then be subjected to high-throughput sequencing on our SOLID platform to obtain global information. Since the human genome contains thousands of nearly identical copies of these elements we use novel computational tools to align these activated LTR elements to the genome. This will tell us from which gene they come from. This will be done in collaboration with our bioinformatician Pierre Cauchy who has devised such methodology.

2. What is the pattern of HL activation in different HL cell lines?
These studies will be performed in several HL cell lines and the differential pattern of LTR activation will be determined. This will also tell us which genes may be aberrantly expressed and whether they are candidates for genes involved in tumour pathogenesis. To test this idea we will compare the list of genes identified by this method with microarray expression data-sets from HL cell lines and control cell lines that are publicly available.

3. Which other LTR element classes are activated in HL cells?
Since *CSF1R* is expressed from a particular class of LTRs (*THE1B)* that come from an ancient infection with one specific virus, we have so far concentrated only on this class. However, it is likley that other types of LTR elements are activated as well. We will therefore use a similar methodology as in our previous study to design generic primers for other LTR elements and use PCR to determine the extent of their activation.

4. Is aberrant LTR ativation found in other cancers, such as acute myeloid leukaemia (AML)?
The molecular reason for LTR activation in HL is two-fold: These cells lost the expression of a repressor that is vital for suppressing LTR expression, but they also express a chronically active transcription factor (NFkB) that normally is only activated when induced. Chronic NFkB activation is also found in other leukemic cell types, such as AML cells. To test whether AML cells also express aberrantly activated LTRs, we will use our primer library designed in **(3)** to test this idea using cell lines first, and if successful, switch to RNA prepared from primary AML cells.
In a different line of LLR-funded experiments we will soon generate microarray expression data from these samples. Once available, the student will mine these data to examine whether genes that play a role in epigenetic silencing of LTRs are differentially expressed in these cells.

Techniques emplyed in this project:
Cell culture, RNA-isolation, cDNA-synthesis, cloning, preparation of 5' and 3' RACE libraries as well as basic and more elaborate bioinformatics methods to analyze genome-wide data.

How are you planning to ensure adequate supervision?

I will meet the student on a regular basis in formal meetings at least once every two weeks. I have an open door policy and I am always available should problems arise.
For day-to-day supervision at the bench and at the computer the student will work closely together with Dr Pierre Cauchy who is both an experimental scientist and a bioinformatician. Pierre is employed on our LLR programme grant and he will train the student in all experimental procedures as well as bioinformatics methods and genome analysis. In addition, he/she will closely interact with Dr Anetta Ptasinska who is the senior postdoc employed on our LLR programme and who is an experienced researcher. My group has 6 postdocs altogether who will be there to help.

Once a week, we have a journal club where we discuss current publications. In addition, the student will also attend regular group meetings that we hold together with the groups of Jon Frampto, Bryan Turner, Ferenc Muller, Paul Badenhorst and Peter Cockerill.

The student will also be fully trained in all health and safety issues relevant to his/her lab work.
The student role.
The student will be fully integrated into my research group. Depending on his/her talent at the bench, the student will perform the more simple experiments him/herself independently once trained. I expect a full engagement of the student in the lab and into our research activities as much as his/her time allows, and if successful, I would want him or her to come back to research later.

Primary Supervisor:	Mark Drayson / Farhat Khanim/Chris Bunce
Project Title:	New use of old drugs in the treatment of myeloma; investigation of mechanisms of action against the malignant clone
Department:	Department of Immunity and Infection and School of Biosciences
Cancer:	Email: f.l.khanim@bham.ac.uk Tel: 0121 414 8680
Discipline:	Cancer Sciences ■ Pathology ☐ Metabolic Medicine ☐ Haematology ■ Infection ☐ Immunology ☐ Anatomy ☐ Endocrinology ☐ Liver & GI Medicine ☐ Evolutionary basis of clinical medicine ☐

Project Outline

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. Affordable, non-toxic treatments that are suitable for even elderly myeloma patients are needed urgently.

Translational research in Birmingham has identified new molecular targets for a combination of two old drugs (progesterone and bezafibrate). As a result, the drug combination is in clinical trials in the UK for adult acute leukaemia and lymphomas, and also in childhood Burkitt's lymphoma in Africa. Utilising a similar drug redeployment strategy, investigation of a library of a hundred existing drugs with diverse clinical use has identified drug combinations (an anti-tapeworm drug and an anti-epileptic) with activities against myeloma cell lines and primary tumour cells from myeloma patients bone marrow. Clinical tests are currently planned and being undertaken here in the UK and in the US with one of these agents. Three consecutive intercalating students have taken the project to the point where we have a good idea of the mode of action of at least one of the drugs. We still need to understand the mode of action of the second drug against myeloma cells. In continuing studies, we would like to understand how the drugs are affecting the mitochondria of the myeloma cells (fission and fusion), the signalling that arises as a result of mitochondrial changes, and the actions of the drugs when myeloma cells are placed in the context of other bone marrow cells and conditions such as hypoxia.

The drugs will be tested for their affect on cell proliferation, survival / apoptosis /autophagy and secretion of M-protein. Investigation into mechanisms of action and molecular targets will include measurement of reactive oxygen species, activation of nuclear transcription factors and cell signalling important in myeloma. An important aspect that will be investigated is how the drugs modify the structure and function of mitochondria and how this relates to the anti-myeloma activity of the drugs. This will be investigated using advanced fluorescence/time-lapse microscopy, immunoblotting and other molecular biology techniques.

How are you planning to ensure adequate supervision?

The student will be co-supervised by Professor Mark Drayson, Dr Farhat Khanim and Prof Chris Bunce. We will have regular meetings to discuss progress and to address any developments in the project. Daily laboratory supervision will be undertaken by Dr Farhat Khanim. Some of the techniques used in the project are undertaken in the Clinical Immunology Service run by Prof Mark Drayson. This will give the student a very useful insight into clinical laboratory techniques to compliment the cell and molecular techniques they will learn in the research laboratory. .
The student role.
Clinical researchers are absolutely essential for successful translation of research findings into the clinic. Hence it is important that intercalating students have a positive research project experience and leave with the motivation and enthusiasm to continue with research. Hence, the student will work within the supportive network of a larger group of post-docs and students, will be encouraged to interact with them, and will be treated as a member of the research team. The project is organised such that the student will be very closely supervised during the first few weeks and semester. Once the student is confident in the lab and with the laboratory protocols, the students are given more independence to organise their own time and design the experiments. The student will be encouraged to read papers and develop their own ideas and hypotheses about the work and design their own experiments. They will also be encouraged to attend national and international meetings to present their work if a relevant meeting/conference comes up. This supervisory approach has been extremely successful with our previous students. Blair Merrick won a poster prize at the International Myeloma Workshop in Paris 2011 and Hannah Giles presented the work at a national student conference 2010 and won third prize for best oral presentation. Our goal is to give the students real experience of research and the importance of the interface between the lab and the clinic.

Primary Supervisor:	Dr. Annette Pachnio and Prof. Paul Moss
Project Title:	Analysis of CMV-specific T cell responses and their role in T cell large granular lymphocytosis (T-LGL)
Department:	School of Cancer Sciences
Contact:	Email: a.pachino@bham.ac.uk Tel: 0121 414 4485
Discipline:	Cancer Sciences [ ] Pathology [ ] Metabolic Medicine [ ] Haematology [X] Infection [ ] Immunology [X] Anatomy [ ] Endocrinology [ ] Liver & GI Medicine [ ] Evolutionary basis of clinical medicine [ ]

Project Outline

Cytomegalovius (CMV), a member of the herpesvirus family, infects about 60-90% of the adult population. The infection is usually asymptomatic in healthy donors, but can cause severe problems in the immune suppressed. With age the T cell response against this virus accumulates and takes up a large space of the immune system potentially limiting the ability of the host to fight other infections.
The CD8 T cells response to CMV has been studied in quite some detail, however, the knowledge about the CD4 T cell response somewhat lags behind. A large study by Sylwester et al. in 2005 showed a broad range of CD4 T cell responses against a great number of CMV-derived proteins and defined a hierarchy of the most frequent and largest T cell responses for both CD4 and CD8 T cells [1]. For CD4 T cells glycoprotein B (gB), part of the viral envelope, was found to be the most frequently recognised antigen. This is not surprising as it has been shown to traffic to the endosome and therefore will be fed directly into the MHC class II pathway to be displayed to CD4 T cells. Nonetheless, to date very few specific epitopes have been defined.Studies in our lab have shown that one known HLA-DR7 restricted gB-derived epitope induces some of the largest CD4 T cell responses observed to date and these expand with age [2]. In addition there is some implication that this extreme expansion may explain the clinical phenomenon of CD4+ T cell large granular lymphocytosis (T-LGL) that has been observed in HLA-DR7 individuals [3].
Aim 1 of this project will be to investigate whether the gB-protein contains other epitopes that elicit such large responses or whether this is solely the case for the known HLA-DR7 epitope. At the same time we would like to screen two other CMV derived glycoproteins that are co-expressed with gB, but do not traffic to the endosome – namely glrcoprotein H (gH) and glycoprotein L (gL). This screening work will be done in healthy lab donors using peptide libraries.
Aim 2 of this project will be to study a cohort of T-LGL patients in collabotation with Dr. Christopher Fox and colleagues in Nottingham. We would like to establish whether there is a link between the large T cells expansions observed in these patients and CMV-infection, especially T cell responses against this virus and the known HLA-DR7 restricted gB epitope in particular. So far this has only been speculated. For this particular epitope we have tetramer reagents available and therefore will be able to identify virus-specific T cell directly *ex vivo*.

1. Sylwester, A.W., et al., *Broadly targeted human cytomegalovirus-specific CD4+ and CD8+ T cells dominate the memory compartments of exposed*

subjects. J Exp Med, 2005. **202**(5): p. 673-85.
2. Crompton, L., et al., *CD4+ T cells specific for glycoprotein B from cytomegalovirus exhibit extreme conservation of T-cell receptor usage between different individuals.* Blood, 2008. **111**(4): p. 2053-61.
3. Garrido, P., et al., *Monoclonal TCR-Vbeta13.1+/CD4+/NKa+/CD8-/+dim T-LGL lymphocytosis: evidence for an antigen-driven chronic T-cell stimulation origin.* Blood, 2007. **109**(11): p. 4890-8.

How are you planning to ensure adequate supervision?

Dr Pachnio is an experienced lab-based scientist and supported by a technician. Together we will provide training in the relevant techniques and daily supervision will be provided. In addition Dr Pachnio will have regular meetings with the student to ensure the support needed to design and execute specific experiments.
The Moss group also have weekly lab meetings the student will be able to attend.

The student role.

The student will be isolating PBMC from peripheral blood samples. These samples will be either from healthy donors within the laboratory or patient samples.
To investigate reactivity of T cells to specific antigens these cells will then be stimulated with peptides derived from the viral proteins specified in the project outline. This will be followed by intracellular cytokine staining and flow cytometry to identify T cells that produce Interferon-· in response to the antigen.

Where possible tetramer reagents will be used to identify virus-specific T cells directly *ex vivo* in combination with antibody panels to further characterise these cells. Those will then be analysed using flow cytometry.

To determine the CMV-serostatus of T-LGL patients recruited plasma will be kept and an ELISA performed to detect CMV-specific IgG antibodies.

During the course of the project the student will learn how to perform these assays independently and how to analyse and interpret the results received.

Primary Supervisor:	Steve Watson
Project Title:	The role of the C-type lectin receptor CLEC-2 and platelets in inflammatory processes
Department:	School of Clinical and Experimental Medicine
Discipline:	haematology, immunity

Project Outline

CLEC-2 is a C-type lectin receptor that is expressed on platelets and, at a low level, on a sub-population of other haematopoietic cells. CLEC-2 is a receptor for podoplanin which is expressed on a wide variety of tissues including lymphatic endothelial cells, lung type 1 alveolar cells, kidney podocytes and inflammatory macrophages. However, it is not present on vacular endothelial cells.
Although there is some evidence that CLEC-2 plays a role in thrombosis, its major role is recognised in development and in tissue maintenance following injury. For example, CLEC-2 deficient mice embryos have blood filled lymphatics in mid gestation due to a failure to separate from the venous endothelial cell layer (Finney et al., 2012). This project the possible role of CLEC-2 in the repair and maintenance of podoplanin-expressing tissues following various challenges such as radiation treatment, exposure to LPS or feeding on a high fat diet (on an ApoE-/- background). The biochemical basis of the defects will also be investigated. Additional support for this project will be obtained from colleagues throughout the College.

Finney, B.A., Schweighoffer, E., Navarro-Núñez, L., Bénézech, C., Barone, F., Hughes, C.E., Langhan, S., Lowe, K.L., Pollitt, A.Y., Mauro-Sa, D., Sheardown, S., Nash, G.B., Smither, N., Reis e Sousa, C., Tybulewicz, V.L.J. and Watson, S.P. (2012) CLEC-2 and Syk in the megakaryocytic/platelet lineage are essential for development. Blood in press

How are you planning to ensure adequate supervision?

The student will work in a multidisciplinary environment with a healthy ratio of postdoctoral fellows to students, with specific help being given as required.

The student role.

The student will be responsible for all aspects of the project including input into experimental goals and design, and undertaking the work.

The student will be expected to undertake a Home Office Course for animal experimentation.

Primary Supervisor:	Dr. Shahrad Taheri
Project Title:	Primary care Research Into Diabetes Evolution (PRIDE)
Department:	School of Clinical & experimental sciences and School of health and population sciences
Contact:	Email: s.m.choudhury@bham.ac.uk Tel: 0121 414 2766
Discipline:	Cancer Sciences [ ] Immunology [ ] Pathology [ ] Anatomy [ ] Metabolic Medicine [ ] Endocrinology [X] Haematology [ ] Liver & GI Medicine [ ] Infection [ ] Evolutionary basis of clinical medicine [ ]

Project Outline

Diabetes mellitus (type 1 and type 2) is a major growing health problem in the UK. It is associated with numerous long term complications including macrovascular (heart disease, stroke, peripheral vascular disease) and microvascular (eye [retinopathy], kidney [nephropathy], nerve [neuropathy]) complications. These complications have an adverse effect on quality of life and are costly to the health service and society. Developing effective interventions for patients with diabetes requires a greater understanding of contemporary patients, and medical and environmental factors.

This project aims to study a population of patients with diabetes within a discrete primary care trust area, South Birmingham Primary Care Trust (PCT), having a diabetes patient population of approximately 15,000. The larger cohort will provide a well characterized population for future studies into diabetes. Subsequently, the project aims to conduct a more detailed study of type 2 diabetes, using more specific validated measures and instruments, in a subset of the cohort population to assess important factors that contribute to diabetes and its macro and microvascular complications. This study will provide important information to the NHS and health professionals aiming to develop effective interventions to prevent and treat diabetes by advancing our understanding of the pathophysiology and care of diabetes.

The aim of the project is to gain a greater understanding of contemporary diabetes across a discrete area; South Birmingham PCT. The project will allow testing of multiple hypotheses important to understanding contemporary diabetes and its complications.
The overall aim of this study is to characterize the diabetes population across a discrete area [South Birmingham Primary Care Trust (SBPCT)] in order to deliver more patient centred initiatives to improve their care.
The broad objectives of the study are to:
1. Approach a large number of patients with diabetes (type 1 & 2) across SBPCT and collect data to get a snapshot of their health status in order to detect those at risk of developing more complications (PRIDE 1). This group will be followed up annually for a further 2 years.
2. Subsequently to conduct a comprehensive assessment in a subset of the cohort population to assess factors affecting patients' lifestyle behaviours, perceived health and well being, cognitive function, sleep, physical activity, and screening for major diabetes complications (PRIDE 2).
3. Conduct a qualitative study to assess patient experiences of type 2 diabetes (PRIDE 3).

How are you planning to ensure adequate supervision?
The student will be asked to attend weekly team meetings and also meet with the supervisor at pre-arranged times. The team consists of researchers, PhD students and statisticians, who will be working on this study and so will be able to help. There is always access via e-mail as well. .
The student role.
The student will take the role of a researcher, helping with patient recruitment and data collection.

Primary Supervisor:	Vivek Dhir and Wiebke Arlt
Project Title:	Zebrafish as a model organism to study sulphation pathways and their role in hyperandrogenism and drug detoxification
Department:	School of Clinical and Experimental Medicine
Contact:	Email: v.dhir@bham.ac.uk
Discipline:	Cancer Sciences ☐ Pathology ☐ Metabolic Medicine ☐ Haematology ☐ Infection ☐ Immunology ☐ Anatomy ☐ Endocrinology ■ Liver & GI Medicine ☐ Evolutionary basis of clinical medicine ☐

Project Outline

The conjugation of a sulphate group (sulphation) to small molecules has an integral and essential role in living organisms. In mammalian physiology, the sulphation system is important for detoxifying drugs, food additives, and toxins from intestinal bacteria or the environment. In humans, defects to the sulphation pathway result in a broad range of clinical phenotypes ranging from neurological disorders, severe bone phenotypes and disorders of androgen synthesis. In both sexes, altered androgen synthesis is linked to type 2 diabetes, heart disease and high blood pressure contributing to premature mortality. Additionally excessive blood levels of the hormone testosterone in females results in hyperandrogenism, and is associated with characteristic features of the polycystic ovary syndrome (PCOS), the leading factor causing infertility. PCOS which affects approximately 5-10% of all women is the most common androgen excess disorder causing not only infertility but also a significantly increased risk of the metabolic syndrome and thus increased adverse risk for long-term health. Dehydroepiandrosterone (DHEA) is the crucial sex steroid precursor in humans; its conversion to active androgens can be prevented by inactivation of DHEA to its sulphate ester DHEAS. This inactivation is catalyzed by DHEA sulfotransferase (SULT2A1) that requires the universal sulfate donor 3′-phosphoadenosine-5′-phosphosulfate (PAPS) for activity. We recently identified a novel disorder manifesting with early onset PCOS that is caused by inactivating mutations in PAPS synthase type 2 (PAPSS2). There are two isoforms of PAPSS which perform a similar function to generate PAPS. Interestingly PAPSS1 cannot compensate for the loss of PAPSS2 in the context of PAPSS2 deficiency. Using a zebrafish model the differential contributions of the two PAPS synthase isozyme in a whole organism model will be investigated. The project will characterise the expression pattern of both *PAPSS* isozymes throughout the **developmental stages of the zebrafish** and reporter constructs driven by the PAPSS gene promoter regions will be used to create **transgenic fish expressing fluorescent reporter genes. In addition** using a fluorescent tissue specific marker, the influence of differential knockdown of the two PAPSS isozymes by specific **morpholino anti-sense oligonucleotides** will be monitored in the living embryo.

How are you planning to ensure adequate supervision?

There will be daily meetings with the student, in addition to a weekly meeting with the wider group, to ensure expectations can be managed. All laboratory based techniques will be taught by experienced members of the lab. The written report will be seen at least once in its entirety before final submission.

.

The student role.

The student will learn and utilise technologies in Molecular biology, whole mount in situ hybridisation, embryo culture and manipulation and, fluorescent microscopy to describe the expression of PAPSS1, PAPSS2A and PAPSS2B in zebrafish through early development. In addition they will begin the functional characterisation of these genes, with a particular interest in the adrenal gland and the liver.

Primary Supervisor:	David Adams and Chris Weston
Project Title:	Vascular adhesion protein-1: a novel regulator of monocyte differentiation
Department:	Centre for Liver Research, School of Immunity and Infection
Discipline:	Cancer Sciences ☐ Immunology x Pathology ☐ Anatomy ☐ Metabolic Medicine ☐ Endocrinology ☐ Haematology ☐ Liver & GI Medicine x Infection ☐ Evolutionary basis of clinical medicine ☐

Project Outline

Background: The prevalence of chronic liver disease in the UK is increasing exponentially and as most liver diseases are incurable the only option for most patients with end-stage liver disease is transplantation. Chronic liver disease is a consequence of dysregulation of inflammatory and/or fibrogenic responses that result in tissue damage. One molecule that is thought to be involved in this process is vascular adhesion protein-1 (VAP-1), a membrane-bound amine oxidase that participates in the recruitment of immune cells into the liver[1]. We have recently shown that VAP-1 plays a key role in the initiation and progression of hepatic fibrosis, and that this process is driven by the nature and extent of the inflammatory infiltrate. Increased levels of a soluble form of this molecule are produced by the liver in patients with chronic liver disease[2] and correlate well with fibrosis stage. The functions of soluble VAP-1 are poorly understood. Recent studies in our laboratory suggest that soluble VAP-1 can modulate the balance between two key players in the maintenance of hepatic inflammation – tissue macrophages and dendritic cells. This is an exciting finding as the fine-tuning of these cell types by VAP-1 has the potential to tip the balance between immune surveillance and tissue remodelling with important implications for the development and progression of chronic hepatitis and fibrosis. We have implicated two signalling molecules, suppressor of cytokine signalling-1 and -3 (SOCS-1 and SOCS-3) in this process and **hypothesise that VAP-1 might regulate monocyte differentiation through these pathways.**

Aims: 1) To identify the effects of VAP-1 activation of monocyte differentiation 2) To define the molecular pathways involved.

Techniques: The project will use well-characterised assays available in our laboratory in which monocytes are differentiated to macrophages or dendritic cells in the presence of specific signals and culture conditions. The role of VAP-1 in these processes will be determined by a) providing soluble VAP-1 or as a cell bound molecule presented by VAP-1 transfectants b) activating VAP-1 by providing specific enzyme substrates or inhibitors. The student will measure the effect of VAP-1 activation on the phenotypic and functional differentiation of monocytes. Finally they will disrupt different signalling pathways including those mediated via SOC-1 and SOCS-3 to determine precisely how VAP-1 mediates this effect. The student will be trained in the following techniques; cell isolation and culture, quantitative real-time PCR, western blotting, flow cytometry, cell proliferation and apoptosis assays, immunohistochemistry and transfection (overexpression and siRNA knockdown).

References

1. ***Activation of vascular adhesion protein-1 on liver endothelium results in an NF-kappaB-dependent increase in lymphocyte adhesion. Lalor PF et al., Hepatology. 2007;45:465-74.***
2. ***CX3CR1 and VAP-1 Dependent Recruitment of CD16+ Monocytes Across Human Liver Sinusoidal Endothelium. Aspinall et al*** ***Hepatology*** ***2010; 51: 2023-2039.***
3. ***Circulating form of human vascular adhesion protein-1 (VAP-1): increased serum levels in inflammatory liver diseases. Kurkijärvi R et al., J Immunol. 1998; 161(3):1549-57***

How are you planning to ensure adequate supervision?

The student will work closely with Dr Chris Weston and a group of scientists studying VAP-1 within the Centre for Liver Research in the IBR. Dr Weston will provide day to day supervision. Prof Adams is based in the CLR and will meet frequently with the student to discuss progress. The student will be expected to present their data at weekly lab meetings and at the CLRs research in progress meetings. The CLR is a research group of more than 30 scientists working within the IBR. It is part of the MRC Centre for Immune Regulation and is thus an outstanding environment in which to carry out research training..

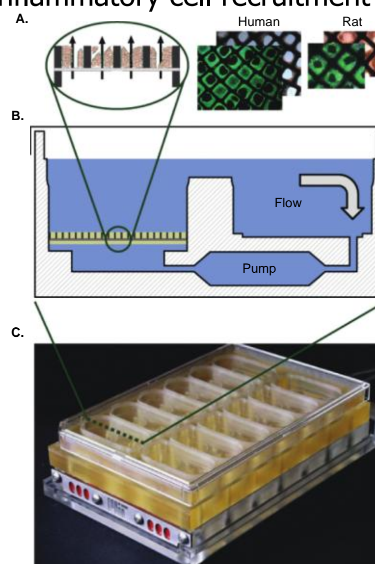
The student role.

The student will have ownership of this project and will carry out the above experiments under the supervision of Dr Weston.

Primary Supervisor:	Dr Patricia Lalor (in collaboration with colleagues at Zyoxel Ltd, Oxford)
Project Title:	Use of perfusion bioreactors to model human liver toxicity
Department:	Centre for Liver Research, Immunity and Infection
Contact:	Email:
Discipline:	Cancer Sciences ☐ **Pathology** ☐ Metabolic Medicine ☐ Haematology ☐ Infection ☐ Immunology ☐ Anatomy ☐ Endocrinology ☐ **Liver & GI Medicine** ☐ Evolutionary basis of clinical medicine ☐

Project Outline

Serious adverse drug reactions to new pharmacological compounds can cause life threatening illness and costly withdrawal of licensed therapeutic compounds. In recent years it has been estimated that up to 25% of reported reactions leading to drug withdrawal in the USA were the result of hepatotoxicity. Thus development of screening tests which incorporate interindividual variation and can assess hepatotoxic effects is increasingly important to big pharmaceutical companies. The liver is composed predominantly of hepatocytes in addition to other cell populations, organised into a complex three-dimensional lobular structure. It's important metabolic, excretory and detoxification functions are carried out by the hepatocytes and facilitated by the structure and cellular constituents of the hepatic sinusoid. Monocultures of hepatocytes or hepatocyte-cell lines are widely used to investigate the biosynthetic, metabolic and detoxification functions of the liver, and similarly monocultured hepatic endothelium is used to quantify inflammatory cell recruitment in models of toxic injury and transplantation. Such in vitro models are useful, but to date these lack the structural and cellular complexity of the hepatic microenvironment. Thus large numbers of animals are currently used for toxicology and disease modelling, and although animal studies can be powerful and informative they have limitations including species-specific differences in gene expression, strain dependent variations and differences in drug transformation capacities between humans and rodents. To address the deficiencies of current methodology, we have been developing in vitro human multi-component cell cultures that provide systems for toxicological screening and investigating human diseases processes *[1,2,3,4]*. Such models are of particular and increasing importance as new EU legislation further restricting use of animals for toxicological and pharmacological screening comes into force in 2012. Our current models require the removal of cell populations from the liver microenvironment and are associated with gradual decline in function over time. We have been collaborating with Zyoxel Ltd to use microperfusion bioreactors in order to generate multicomponent culture systems which recreate hepatospecific functions in a stable, perfused system for long term assays (see Figure to left).



To date there is little or no data on the applicability of the system to human cells and no studies have used cocultures of hepatocytes with non-parenchymal cells to improve functionality.
In the current proposal we wish to optimise use of the Zyoxel system with primary human cells and to test their applicability for hepatotoxicity testing and maintenance of hepatospecific functions.

Thus we shall use functional biosynthetic assays, viability assays, electron and fluorescent microscopy and standard histochemistry to determine the performance of hepatocytes in perfusion bioreactors compared to traditional monoculture. We will also assess whether coculture with non-parenchymal cells improves outcome. We will then challenge the system using hypoxia, proinflammatory cytokines and pharmacological reagents known to be associated with adverse toxicological effects (acetaminophen, troglitazone, chloramphenicol etc) in order to determine the viability of the system as a pharmacological screening tool.

Key references :

1. Bhogal RH, Weston CJ, Curbishley SM, Bhatt AN, Adams DH, et al. (2011) Variable responses of small and large human hepatocytes to hypoxia and hypoxia/reoxygenation (H-R). FEBS Lett 585: 935-941.
2. Bhogal RH, Hodson J, Bartlett DC, Weston CJ, Curbishley SM, et al. (2011) Isolation of primary human hepatocytes from normal and diseased liver tissue: a one hundred liver experience. PLoS One 6: e18222.
3. Edwards S, Lalor PF, Nash GB, Rainger GE, Adams DH (2005) Lymphocyte traffic through sinusoidal endothelial cells is regulated by hepatocytes. Hepatology 41: 451-459.
4. Holt AP, Haughton EL, Lalor PF, Filer A, Buckley CD, et al. (2009) Liver myofibroblasts regulate infiltration and positioning of lymphocytes in human liver. Gastroenterology 136: 705-714
.5. Liaskou E, Karikoski M, Reynolds GM, Lalor PF, Weston CJ, et al. (2010) Regulation of mucosal addressin cell adhesion molecule 1 expression in human and mice by vascular adhesion protein 1 amine oxidase activity. Hepatology.

How are you planning to ensure adequate supervision?

Dr Lalor is a well established PhD supervisor for both clinical and basic science candidates and module coordinator for a Final year module on the BMedsci course and thus has several years experience of supervision of both postgraduate and undergraduate research projects. The student would integrate into the larger Centre for Liver Research environment and thus have interactions with students and established clinical and scientific researchers at all stages of their research careers. Experienced technical staff are also on hand to aid with laboratory skills training as necessary. Weekly meetings would be scheduled with the academic supervisors and where possible monthly feedback meetings will be arranged with the pharmaceutical co-supervisor form Zyoxel. All students in the group are expected to attend and to present at research in progress meetings thus ensuring wide exposure to translational research data and opportunities to gain presentations skills.
.

The student role.

The student will be expected to perform experimental analyses independently upon completion of in-house training and acquisition of technical competence. The will be expected to keep contemporary detailed laboratory records and develop their analytical and presentation skills by presentation of their data at meetings with the supervisory team and wider research group. Upon guidance they should assimilate the relevant specialist literature and generate a review of the field for the introduction of their project dissertation. They will have many opportunities to interact with the other researchers in the lab and daily support will be available as necessary.

Primary Supervisor:	Phil Newsome
Project Title:	Mesenchymal stem cell action and homing in liver injury
Department:	School of Immunity and Infection
Cancer:	Email: p.n.newsome@bham.ac.uk Tel: 0121 4145614
Discipline:	Cancer Sciences ☐ Immunology ☐ Pathology ☐ Anatomy ☐ Metabolic Medicine ☐ Endocrinology ☐ Haematology ☐ Liver & GI Medicine ■ Infection ☐ Evolutionary basis of clinical medicine ☐

Project Outline

There are two major types of adult stem cell found in the BM; haematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). MSCs comprise a cell population characterised by their ability to adhere to plastic, the expression of surface markers including CD73, CD90, and CD105, ability to differentiate into adipocytes, chondrocytes and osteoblasts and latterly ability to immunosuppress. More recently data have demonstrated that MSCs can reduce liver injury by suppressing inflammation, which increasingly is felt to be their dominant mode of action.

We have a novel flow sorting protocol to isolate a purified population of mouse MSCs from murine bone marrow based on their expression of Sca-1 and PDGFRα. PαS cells have augmented growth potential, robust tri-lineage differentiation and potent immunosuppressive actions compared to conventional plastic adherent MSCs.

The purpose of the project will be to work alongside an established team in the lab to study the action of mouse MSCs in models of liver injury. This will build on data already generated on their action in liver injury in my lab.

The student will focus on the factors controlling the trafficking of MSCs to injured liver, and then determine the impact of altering their hepatic migration.

Two models of liver injury will be used: (i) Carbon Tetrachloride and (ii) OVA-BIL transgenic mice tolerant to OVA, will receive adoptive transfer of OVA-specific CD4+ and CD8+ T cells to create immune-medaited biliary-centred damage.

Tissue binding experiments will identify factors responsible for adhesion of MSCs to liver tissue. The role played by each receptor will be determined using in vitro flow-based adhesion assays and intra-vital microscopy. Either wild type RFP^+ MSCs or adhesion molecule knockdown RFP^+ MSCs (which are also GFP^+ as the result of successful lentiviral transduction), will be infused into mice prior to the establishment of liver injury. The overall amount of liver injury will be quantified and compared between the two groups. We predict that disabling the hepatic migration of MSCs will lead to increased liver damage, as MSCs need to migrate to the injured liver to exert their protective effect.

The successful student (live project~) will be eligible for a CORE Falk bursary.

How are you planning to ensure adequate supervision?
There will be weekly meetings with me. In the lab they will be supervised on a daily basis by my post-doc, PhD students and technician who are working directly on mesenchymal stem cell projects.
The student role.
The student will participate in the isolation and culture of mouse MSCs. They will also define the adhesion molecules responsible for adhesion and migration to liver endothelium in a range of static and flow-based assays. They will assist the team with the in vivo mouse studies, and then analyse the mouse tissue afterwards. This will include analysis of liver damage, non-invasive imaging of MSC distribution within the mouse and analysis of inflammatory cell sub-sets within the liver. An animal licence will not be required. The student will be exposed to a range of laboratory techniques including flow cytometry, cell sorting, cell culture, IVUS whole animal imaging, immunohistochemistry and tissue disaggregation.