Development of multi-SNP pharmacogenetic predictors of antiepilepsy drug efficacy
Supervisors: Dr Michael Johnson (IC) and Professor David Balding (UCL)
Mentor: Aroon Hingorani (UCL)

Background: Epilepsy has huge public health significance, costing the UK over £1 billion/year. Approximately 30% of patients with epilepsy continue to have seizures despite optimal medical therapy. Failure to achieve seizure control in patients with epilepsy is associated with increased physical and psychosocial morbidity, as well as an increased risk of death. It is not currently possible to predict the success of seizure control with antiepilepsy drug (AED) treatment in an individual patient. The identification of genetic markers that provide prediction in an individual patient of their chance of seizure control with AED therapy would be of major clinical and socioeconomic value. A major obstacle to the development of genetic tests for drug efficacy has been the absence of suitable information-dense datasets that can enable the discovery of an optimized panel of genotypes with stable risk estimates for use in clinical practice. Critically, the development of predictive models for drug response outcome require prospective datasets of sufficient duration so as to record clinically relevant outcomes. The considerable expense and time involved in the ascertainment of such datasets has resulted in all previous attempts in epilepsy pharmacogenetics (PGx) being undertaken in an inappropriate retrospective case-control setting. In contrast, over the past five years, we (MJ) have systematically established the single largest prospective epilepsy PGx cohort worldwide (n=1000). Through collaboration (T O'Brien, University of Melbourne), we are now able to combine this with a second European ancestry prospective epilepsy PGx cohort (n=450). All samples have been fully genotyped (Illumina 660Q BeadChip) providing a unique, "ready to go" dataset of integrated clinical and genomic information specifically to inform the development of predictive tests of AED response in newly treated epilepsy. During Year 2 of the project, we will gain access to a second prospective, European ancestry, genotyped epilepsy PGx cohort (n=500 R Kalviainen, University of Kuopio), allowing validation of the derived predictive PGx models in a completely unseen cohort of epilepsy patients.

Plan for Clinical Training Fellowship: Under careful clinical and statistical guidance, the fellow will apply the latest statistical methods to the analysis complex PGx datasets to derive multi-SNP predictors of AED response. We (the co-supervisors to this research fellowship) have recently been awarded a research grant (MRC Ref: G0901388) to develop statistical methods for efficacy PGx using GWAS data. The PhD student will apply and develop the latest advances from this major research effort to our unique prospective epilepsy PGx dataset, with the key aim of rapid translation to the specialist healthcare.

Translational benefits and skillset acquisition for Clinical Pharmacology: The era of genomic medicine requires that medical graduates are knowledgeable in the interpretation of genomic information (http://www.publications.parliament.uk/pa/ld200809/ldselect/ldsctech/107/107i.pdf). If doctors are required to use genomic tests in making diagnoses or treatment decisions, training in genomic medicine should be a core competency. This project will promote the development and translational implementation of genomic medicine in clinical neurology, and provide a level of research training that will allow the research fellow to take a future leading educational role in ensuring medical graduates are fully trained to succeed in modern, genomics-informed healthcare systems.
**Key milestones: Year 1:** Under appropriate clinical and statistical supervision, the fellow will first prioritize Bayesian statistical methods for efficacy PGx, with the aim of deriving models for predicting drug response outcome based on clinical and genomic data and their joint interactions. **Year 2:** The developed models will be validated in an independent prospective epilepsy PGx cohort provided in collaboration by Professor Kalviainen, University of Kuopio (see above). **Year 3:** The fellow will design a web-based pharmacogenetic decision support tool with licensed genetic testing for AED efficacy for treating physicians worldwide, allowing real-time prospective validation of the derived predictive models. At 33 months all experimental work will be completed in order for the student to have sufficient time to finish writing and submission of her/his thesis before the end of the 36 month PhD period. The output will be an MRes PhD.
Harnessing Genomic Advances for Drug Discovery and Target Validation

Supervisors: Professor Aroon Hingorani (UCL) and Professor Liam Smeeth (LSHTM)
Mentor: Professor Mark Caulfield (QMUL)

Background: There is widespread recognition that the current model of drug development is becoming unsustainable (1). Overall, the output of new molecular entities is growing linearly (at a slower rate than would be expected from the advances in basic sciences) while development costs are increasing exponentially. The major problem is not the paucity of drug targets but rather the low probability that any of the vast number of new molecular entities will become a successful therapy. The major difficulty recognised by the Cooksey report is in the validation of therapeutic targets using the existing investigative tools. In some disease areas (e.g. cardiovascular disease) drug development is perceived as stagnating, while genomics has been advancing at a previously unparalleled pace. Despite this it has been questioned whether genomic advances can be successfully translated into improved health care.

The established view of the interface between genomics and therapeutics is in the development of genotype-based tests to stratify disease risk or drug response (pharmacogenetics). However, a further, complementary application is to exploit the unique properties of genotype (assigned at random and fixed through life) to identify new drug targets through genome wide studies in common diseases, and to utilise variants in genes encoding existing targets as a type of natural randomised trial to help delineate the likely effects of modifying these targets pharmacologically (2,3, 4).

Proposal: In this research theme, trainees will exploit advances in complex disease genomics to improve the efficiency and enhance the likelihood of success of drug development programmes by. Specific possible projects include:
(1) Critically evaluating the use of newly emergent disease relevant alleles as tools to stratify risk of common diseases to identify subjects at higher risk in whom preventative therapies could be more cost-effectively targeted;
(2) Critically evaluating common variants in genes encoding proteins involved in drug absorption, distribution, metabolism and excretion to help develop pharmacogenetic tests to the same standards as non-genetic screening and diagnostic health technologies;
(3) Exploiting findings from genome wide studies as a new source of drug targets and utilising focused genetic studies of drug targets as a new tool for target validation.

Translational benefit and skill set acquisition for clinical pharmacology: The work in this theme will be underpinned by the expertise of the supervisors in all three areas, their ongoing work in discovering the genes influencing blood pressure (5), blood lipids and other circulating biomarkers (6), ECG variables as well as pre-eclampsia, as well as the large, highly phenotyped populations studies and case collections hosted at the participating institutions.

The theme should help develop a new cadre of clinician-scientists equally comfortable in statistics, epidemiology, bioinformatics, genetics and clinical pharmacology who will be needed to drive innovation in drug development.

1. Munos B. Lessons from 60 years of pharmaceutical innovation. Nat Rev Drug Discov. 2009; 12; 959-68
Molecular imaging of pulmonary arterial hypertension
Supervisors: Professors Martin Wilkins and Eric Aboagye (Imperial College)
Mentor: Emma Baker (SGUL)

Background: Pulmonary arterial hypertension (PAH) is a heterogenous condition characterised by raised pulmonary vascular resistance due to vascular remodelling. Current treatments are based on reducing vascular resistance by relaxing vascular tone. The development of medicines that target directly the vascular remodelling (eg receptor tyrosine kinase inhibitors) raises new challenges. Measuring the response of the pulmonary circulation to acute dosing to assess efficacy and enable dose selection is not an option. While the longer term haemodynamic and functional clinical endpoints remain important, their use for screening potential therapeutic candidates is compromised by the time needed to measure a response (3 months or longer) and the numbers of patients required to have confidence in any change observed.

Positron emission tomography (PET) is used in oncology to interrogate a number of pathological processes relevant to PAH – for example, proliferation, apoptosis, angiogenesis and inflammation. In PAH, it offers the promise to refine the diagnosis of PAH at the molecular level, specifically to subcategorise patients according to their underlying pathology. It also has the potential to follow the biochemistry and early effects of a drug-tissue interaction with a relatively quick readout signal (days/weeks rather than months) in a small number of patients, provide proof-of-mechanism of action and permit dose selection for future studies.

Hypothesis I: PET imaging of cell proliferation using F-3′-deoxy-3′-fluoro-L-thymidine (F-FLT) as a radiotracer can be used (1) to stage patients with PAH and (2) follow response to treatment.

Hypothesis II: PET imaging of integrin receptor αvβ3 status using [18F]fluciclatide (F-Angio) as a radiotracer can be used (1) to stage patient with PAH and (2) follow response to treatment.

Plan for Clinical Training Fellowship: Year 1: Familiarisation with animal models of PAH and methodology for assessing haemodynamic and vascular pathology; principles of PET and develop experience with radiotracers in animal model.

Year 2: Begin to examine the effect of a candidate tyrosine kinase inhibitor (sunitinib) on the vascular proliferation response in a validated animal model of pulmonary hypertension. Proliferation as measured by FLT and FAngio will be correlated with cell proliferation and vascular remodelling as measured using histochemical techniques.

Year 3: Image patients with PAH and correlate data with stage of disease (as assessed by WHO class, 6 minute walk distance). The use of PET as a bridging biomarker to human studies will be investigated in patents before and after treatment with either approved therapies or in conjunction with a clinical trial of a tyrosine kinase inhibitor in PAH.

Translational benefits and skill set acquisition for Clinical Pharmacology: The Fellow will learn the advantages and limitations of animals models, develop skills with the use of radiotracers for in vivo studies, be involved in the design and conduct of a PET study in humans and be exposed to the modelling and analysis of image data. The Fellow will learn the value of bridging biomarkers in early phase clinical studies.
Effects of Zoledronic Acid on Arterial Stiffness and Arterial Calcification

Supervisor: Professors P Chowienczyk and Albert Ferro (KCL)
Mentor: Liam Smeeth (LSTMH)

Background: Arterial stiffness is one of the most important predictors of cardiovascular morbidity and mortality.\(^1\) Recent data suggest that arterial calcification is the main cause of increased stiffness. At present there is no treatment to prevent or reverse arterial stiffening. However, in animal models bisphosphonates have powerful effects in preventing vascular calcification.\(^2\) In the Health Outcomes and Reduced Incidence with Zoledronic Acid Once Yearly (HORIZON) Recurrent Fracture Trial, zoledronic acid, administered after a fragility fracture, reduced new fractures and produced a surprising reduction in all cause mortality (hazard ratio 0.72 95% CI 0.56 to 0.93, \(P<0.01\)).\(^3\) The trial was not powered for mortality as primary outcome and the cause of the reduction in mortality was not determined. However, there was a trend towards reduction in cardiovascular death (\(P=0.1\)) consistent with a beneficial effect of zoledronic acid on arterial calcification and stiffness (which were not measured). The aim of this project will be to perform a randomised clinical trial to examine effects of zoledronic acid on arterial stiffness and calcification. In a co-twin case control study, postmenopausal female MZ twins (n=200) from the Twins UK cohort (all of whom have regular bone density measurements and in whom arterial stiffness has been measured) in whom bone density falls into the osteopenic range and arterial stiffness is elevated will be randomly allocated (one twin of each pair receiving active treatment) to annual infusions of zoledronic acid/placebo. Aortic calcification will be measured by CT using a modified score developed to quantitate aortic calcification.\(^4\) Assuming annual progression of PWV in excess of 200 mm/s per year, and a standard deviation of repeated measures of PWV below 600 mm/s\(^5\) this will give ample power to detect any regression of PWV in the active group relative to the placebo group after one year (assuming stability or progression within the placebo group).

Plan for Clinical Training Fellowship: The fellow will join the project at the later stages of planning and be involved in detailed design and submission for regulatory approval (year 1). During year 2 they will play a major role in managing the trial, together with a research assistant, nurse and vascular technician (for which funding is secured). They will gain experience of vascular measurement and imaging. The first year of follow-up will be completed early in year 3, allowing the remainder of the year for data analysis and write-up for the PhD.

Translational benefits and skillset acquisition for Clinical Pharmacology: The trial will provide “hands-on” experience in practical aspects of clinical trial design, ethical and regulatory approval and execution whilst addressing a novel and potentially important therapeutic target for preventing cardiovascular disease.

Sex differences in endothelial function and protection against cardiovascular disease: role of EDHF

Supervisors: Dr. Adrian Hobbs (UCL) & Prof. Amrita Ahluwalia (QMUL)
Mentor: Prof. Raymond MacAllister (UCL)

Background: Pre-menopausal women enjoy a relative protection from CVD when compared to age-matched male counterparts and post-menopausal women; this cardioprotective phenotype is though to be due, at least in part, to ovarian hormones. A number of targets, pathways & mediators have been proposed to play a role in mediating these beneficial effects, but each of these appears to be underpinned by a common mechanism: an oestrogen- induced or enhanced activation of the endothelium. This counteracts the changes in endothelial physiology (‘endothelial dysfunction’) that are instrumental in precipitating the vascular inflammation that is an early, crucial event in the pathogenesis of many ischaemic cardiovascular disorders (e.g. atherosclerosis, myocardial infarction & stroke). Currently understanding promotes the thesis that oestrogens up-regulate the synthesis, release and activity of protective endothelial factors whilst concomitantly suppressing the influence of pathogenic mediators. Indeed, a significant proportion of this anti-inflammatory activity has been attributed to alterations in nitric oxide (NO) bioactivity. However, our preclinical work has revealed that an alternative cytoprotective, endothelium-derived mediator, termed EDHF (endothelium-derived hyperpolarising factor) may underlie the female cardioprotective phenotype. However, the clinical pharmacology and importance of EDHF in the human vasculature has yet to be established. A greater understanding of the mechanisms that underlie the beneficial effects of female sex steroids and EDHF may, therefore, identify novel strategies that could be used to reduce the incidence and severity of cardiovascular disease.

Hypothesis: ‘EDHF underlies the female vasoprotective phenotype in humans’

Plan for clinical training fellowship: The research fellow will conduct in vitro and in vivo studies in animal models and healthy volunteers, exploring the role of EDHF in vascular function and in susceptibility to ischaemia-reperfusion (I/R) injury in males & females. The animal studies will centre on a transgenic mouse line which the supervisors have generated (the ‘EDHF’ mouse) that relies principally on EDHF to maintain cardiovascular integrity. These animals will be employed to explore specific aspects of cardiovascular (patho)physiology, including vascular reactivity, platelet & leukocyte function, and I/R injury. These studies will be complemented by classical functional pharmacological (organ bath) studies in which the role of EDHF in endothelium-dependent dilatation will be evaluated in murine & human vessels of both sexes. Clinical correlates for this work will be provided by investigations in healthy volunteers using forearm blood flow as an index of vascular function, including a model of I/R injury. This methodology is well-established at both UCL and Bart’s Medical School, and has been used successfully to translate observations from pre-clinical work to the human cardiovascular system. Ethical permission is already in place.

Translational benefits and skill set acquisition for Clinical Pharmacology: The long term objective of the project would be to define the importance of EDHF in the regulation of cardiovascular function in humans, and delineate the sex differences that underlie the cardioprotective phenotype of pre-menopausal women. Such data would add value in terms of identifying novel strategies for combating CVD, and also for the clinical use of hormone replacement therapy. In addition to these research/translational outcomes, this multi-disciplinary project will ensure a high quality Clinical Pharmacology training programme directly relevant to human
cardiovascular physiology and disease, encompassing a solid grounding in basic science, *in vivo* models and clinical studies.

**Key milestones:** **Year 1.** The fellow will register for a PhD and obtain a HO animal licence. They will also attend an MRes/MSc in Clinical & Experimental Medicine, receiving generic and transferable skills. The fellow will gain experience and expertise in *in vitro* pharmacology and models of CVD, and initiate pre-clinical studies. **Year 2.** The fellow will complete the *in vitro* and *in vivo* pharmacological investigations and learn the technique of forearm plethysmography to enable the clinical studies to begin. **Year 3.** The fellow will undertake evaluation in healthy volunteers and write up the investigations as a PhD.
Supervisors: Dr Nicholas Simmonds, Professor Margaret Hodson, Dr Bill Cookson (Imperial).
Mentor: Dr Emma Baker (SGUL)

Background: Cystic fibrosis (CF) is the most common, lethal, inherited disease in Caucasians, affecting around 1 in 2,500 individuals. It is caused by mutations in the cystic fibrosis transmembrane regulator gene (CFTR). CFTR functions as a cAMP-dependent chloride channel in epithelial cells and regulates other membrane transporters. Mutations that impair function of CFTR disrupt chloride, sodium and water homeostasis in the lung, pancreas and other organs and result in increased viscosity and reduced clearance of secretions. This initiates a vicious cycle of infection and inflammation that causes chronic organ damage. Most CF patients are disabled by and die prematurely of lung disease with median life expectancy of 38 years. A key goal of treatment for patients and physicians is prolongation of survival.

Although CF is caused by mutations in a single gene, the phenotype varies widely. This is not explained by different effects of diverse CFTR mutations on protein function, as genotype-phenotype correlations are poor. The observation that CFTR phenotype is more closely correlated in monozygotic than in dizygotic twins or siblings indicates that modifier genes may influence phenotype. To date, a candidate gene approach has provided some evidence to support this hypothesis. Significant allelic and genotypic associations with severity of lung disease were seen for transforming growth factor β1 and mannose binding lectin 2 and gene modifiers of liver disease and diabetes have also been identified.

Limitations of these studies include the candidate gene approach, which does not allow discovery of novel disease pathways, and use of intermediate phenotypes such as lung and liver disease rather than survival. The aim of the proposed study is to used genome-wide association techniques to identify heritable quantitative traits that predict prolonged survival (≥40 years) in CF patients. This has important implications for predicting prognosis and stratifying therapy for CF patients.

Plan for Clinical Training Fellowship. In this project the research fellow will work with CF centres in an established network across Europe to collect phenotypic information and blood samples from CF survivors (≥40 years) and from a control group of CF patients aged 18-23 years matched by centre. They will perform genome-wide SNP genotyping for CF survivors and controls. They will learn about quality control in genome wide association studies. They will use genetic analysis software to test for associations between SNPs and survival use gene maps to identify areas of linkage and predict functional consequences.

Translational benefit and skill set acquisition for clinical pharmacology. The long term objective of this project is to identify gene modifiers of survival in cystic fibrosis that could guide individualised treatment for patients and generate new targets for therapeutic intervention. In addition to these translational outcomes this project will provide training in conducting clinical studies in human volunteers and working in large networks as well as in laboratory techniques and data analysis.

Key milestones: We anticipate that ethical approval will be in place and many participating centres will be recruited at the start of the project. Year 1. The fellow will receive research training through the MRes at St George’s in generic research skills and genetics. In their research time they will contribute to data and sample collection, liaise with centres to promote recruitment and develop a database of participants and phenotypes. They will present their phenotypic data at a national/international meeting. Year 2. They will receive specific training in and
Perform genome-wide SNP genotyping, then will use genetic analysis software to look for associations between SNP and survival in CF. **Year 3.** They will complete their analysis, present their data and write their thesis for submission for PhD before the end of the 36 month period. The output will be an MRes PhD.
Phenotyping long-QT syndromes

Supervisor: Prof Andy Tinker and Dr Pier Lambiase (UCL)
Mentor: Mark Caulfield (QMUL)

Background: Heart rhythm disorders are an important cause of death and morbidity in clinical medicine. For example, sudden death due to ventricular arrhythmia, may account for up to 11% of unexpected deaths [1]. There are a number of hereditary diseases leading to arrhythmia and one such disease is the long QT syndrome (LQTS). It is characterised by prolongation of the QT interval on the ECG and this predisposes the individual to torsade-de-pointes and subsequent sudden death due to ventricular fibrillation. There has been substantial progress in understanding the molecular basis of these syndromes. From this work it is apparent now that there is a vast pleiotropy of causation of what appears to be a single clinical entity [2]. This contrasts sharply with cystic fibrosis and CFTR where a single mutation in a single gene accounts for 70% of cases. Specifically, mutations have been identified in a number of genes encoding cardiac Na+ channel, K+ channels and their ancillary proteins. The majority of cases are associated with mutations in the genes encoding the KCNQ1 K+ channel and the auxiliary subunit KCNE1 (LQT1 and LQT5 respectively), the HERG K+ channel (LQT2) and SCN5A (LQT3). In general there are loss of function mutations in the K+ channels and a gain of function in the Na+ channel mutations. Moreover, the underlying molecular pathology is diverse. Initial studies focussed on the electrophysiological consequences of the ion channel mutations in these syndromes. However it has become clear recently that channels can fail to traffic to the plasma membrane [3, 4].

Drug therapy with beta blockers is known to be effective in this condition; however there have only a few attempts to target drug therapy to the underlying channel mutation and/or disease mechanism. The possibilities for mutation specific therapy are actually quite broad. Thus gain of function mutations in Na+ channels might be managed with agents known to block these channels [5]. It is known that KCNQ1 is particularly responsive to ß-adrenergic stimulation and thus ß-blockers are particularly effective in LQT1 [6]. Specific biophysical defects in HERG can be paradoxically overcome by increasing the extracellular K+ concentration. The delivery of channels to the plasma membrane can be facilitated by the use of chemical chaperones and specific drugs [7]. Nonsense mutations might be overcome by agents known to promote read through and one such agent is in use in clinical trials [8, 9]. Finally, specific agents in LQT5, such as fenamates and stilbenes, might be efficacious[10].

Hypothesis:- Gene and mutation specific therapy is potentially advantageous in the hereditary long QT syndromes. Aims are to
1. To characterise disease mechanisms in families with the hereditary long QT syndrome attending the UCL SADS clinic.
2. To determine in-vitro the optimal pharmacological strategy to correct the defective pathology.
3. To explore the possibility of tailoring specific pharmacological therapies in families with specific ion channel mutations.

Experimental Plan: Currently, it is possible to identify disease causing mutations in 50% of long QT cases. Families who have suffered a sudden arrhythmic death (SADS) & cardiac arrest survivors are screened in the UCL SADS clinic for clinical evidence of long QT syndrome and other ion channel disorders such as Brugada
syndrome. The clinic currently sees 70 new referrals a year and a diagnosis of an ion channel disorder is made in 40% of cases. Each case undergoes mutation analysis for recognised ion channel mutations. We will introduce these mutations into cDNAs encoding the relevant channel. We will use a series of cellular assays to understand the molecular pathogenesis [11, 12, 3] of the disease and assay various pharmacological approaches to correct the underlying abnormality. If effective we would seek ways to intervene in a similar manner in the patients.

Reference List

Monitoring the Annexin A1 pathway in circulating leukocytes in myocardial infarct

Supervisors: Prof Mauro Perretti & Dr Steffen Petersen (QMUL)
Mentor: Prof Phil Chowiencyz (KCL)

Background: Annexin A1 is an effector of endogenous anti-inflammation, that is the complex interactions of several pathways and mediators operative in our body to assure regain of tissue homeostasis after an acute inflammatory episode including post-ischaemic events. Human circulating granulocytes are abundant producers of Annexin A1 and also bear its specific receptor (termed FPR2). Autocrine and paracrine mechanisms of Annexin A1 secretion and activation of its anti-inflammatory receptor contribute to keep the extent of neutrophil activation and migration under check [NAT REV IMMUNOL 9: 62, 2009]. The pharmacological potential of this research may be through the development of small and stable peptides, as well as new chemical entities, which would bind FPR2 mimicking the actions of Annexin A1 [BR J PHARMACOL 158: 936, 2009]. Following over 20 years of basic research, we are now ready to exploit this pathway in human disease and have generated the following pilot observations:

1. Granulocytes of patients suffering from giant cell arteritis (GCA) display high levels on both Annexin A1 and FPR2 on their cell surface, as assessed by flow cytometry. Moreover, real-time PCR revealed strong induction of both genes in granulocyte extracts.

2. Compared to healthy controls, patients suffering from other rheumatic disease (e.g. RA) have higher protein (but not gene) expression of either Annexin A1, FPR2 or both.

3. A very recent study reports higher Annexin A1 expression in neutrophils of patients suffering from coronary artery disease [Metabolism, Oct 20, 2009, epub]. Thus, we postulate that increased expression of Annexin A1 (and FPR2) may represent a frustrated attempt of the host to check on aberrant cell activation; Annexin A1 (and FPR2) could be used as a novel biomarker to stratify disease status and monitor patients’ responsiveness.

Plan for Clinical Training Fellowship: In this project, the research fellow would conduct in vitro and ex vivo studies of patients suffering from myocardial infarct divided into 3 groups of patients with acute coronary syndromes (ACS): 1) ST segment elevation myocardial infarction (STEMI), 2) Non ST segment elevation myocardial infarction (NSTEMI) and 3) Unstable Angina. Levels of Annexin A1 and FPR2 expression on circulating leukocytes will be determined with a validated whole blood staining protocol and FACS analysis. Moreover, the fellow will monitor other cell specific (e.g. CD3, CD16 and CD14) and activation (e.g. CD62L, CD11b, CD11a) markers. Plasma levels of classical cytokines, Annexin A1 and SAA will be measured by ELISA. Total and specific cell numbers will also be quantified. We plan to perform cardiovascular magnetic resonance imaging (CMR) in 10 patients in each ACS category and in age- and sex-matched healthy controls. This number of 10 patients would be sufficient to detect significant differences as informed from studies completed in GCA and RA patients. Furthermore, We propose a comprehensive protocol quantifying global left ventricular mass and function, myocardial oedema, microvascular obstruction, myocardial necrosis and myocardial blood flow.

The data obtained with this initial yet systematic characterization will then inform on specific functional data to be produced, with the functional model being determined by the leukocyte type mostly altered in its Annexin A1 and FPR2 profile compared to healthy controls. Example of possible functional assays, all validated in our
laboratories, are neutrophil or mononuclear cell adhesion and transmigration, chemotaxis, interaction with endothelial cell under flow.

**Translational benefits and skillset acquisition for Clinical Pharmacology:** The long-term objective of the project would be to define markers of leukocyte activation in the context of cardiac disease. These initial data will likely overspill into further clinical analyses where the profile of Annexin A1 and FPR2 on white blood cells will be monitored longitudinally, perhaps also associated with therapy efficacy. Assessment of the pattern of expression of FPR2 in a patients’ subset can also guide the application of annexin A1 mimetics currently under development through a collaborative project with Unigene Corp (Fairfield, New Jersey; $1.2M investment).

**Key milestones:**

**Year 1:** the fellow will be participate in the MRes in Vascular Mechanisms in health and disease within the William Harvey Research Institute receiving generic and transferable skill programmes and register for a PhD. Fellow will be trained in techniques for *in vitro* cell biology and laboratory assays, flow cytometry and for *clinical* measurements of blood vessel reactivity, and will begin healthy volunteer studies. It is expected that the fellow will present work at a national meeting derived from the first year of studies by the midpoint of year 2.

**Year 2:** fellow will continue studies of Annexin A1 and FPR2 levels in patients and healthy volunteers. **Year 3:** student will finish up healthy volunteer and patient studies and be expected to present work at a national/international meeting. At 33 months all experimental work will be completed in order for the student to have sufficient time to finish writing and submission of her/his thesis before the end of the 36-month PhD period. The output will be an MRes PhD.