University of Glasgow | College of Medical, Veterinary & Life Sciences

# Head of College Scholars List Scheme

## Summer Studentship

## Report Form

This report should be completed by the student with his/her project supervisor. It should summarise the work undertaken during the project and, once completed, should be sent by email to: jill.morrison@glasgow.ac.uk within four weeks of the end of the studentship.

1. Student

   Surname: Carberry Forename: Jaclyn

   E-mail address: 1101472c@student.gla.ac.uk

2. Supervisor

   Surname: Kennedy Forename: Simon

   E-mail address: Simon.Kennedy@glasgow.ac.uk

3. Research Project Report

   3.1 Project Title (maximum 20 words):

   i) Mast cells and vein graft disease.

   ii) The impact of PVAT on AMPK pathways and VSM relaxation in health and disease.

   3.2 Project Lay Summary (copied from application):

   NB- due to delays in obtaining an extension of Ethical Approval for the study of human EPC and plasma oxLDL, the aims of the project have change from the original submission. We are now investigating the location of mast cells within mouse vein grafts using an immuofluorescence protocol as outlined below. This work will contribute to a

study on how mast cells influence vein graft hyperplasia and which we will shortly submit for publication.

3.3 Start Date: 02/07/13 Finish Date: 22/08/13

3.4 Original project aims and objectives (100 words max):

To use immunofluorescence to stain mast cells in vein grafts from mice. These are grafts from normal, wild-type mice and mice which are genetically deficient in mast cells. In some experiments, mast cells were reconstituted in mast cell deficient mice either systemically or locally around the vein graft. Based on previous data using Toluidine Blue staining, we anticipated finding mast cells in wild type grafts and mast cell deficient mice which had been reconstituted with mast cells locally.

3.5 Methodology: Summarise and include reference to training received in research methods etc. (250 words max):

**Mast Cell Staining**

Location of mast cells within grafts was identified using Avidin Texas red conjugate (Invitrogen). The stain was added to de-waxed slides at a dilution of 1:250 in PBS and incubated overnight. Detection was by means of a fluorescence microscope (Biorad Radiance 2100 on a Nikon TE300) with 488nm line of Argon ion laser excitation, emission 515nm, and 543nm line of Green He/Ne laser excitation, emission 590nm.

**Small vessel wire myography**

Following dissection and preparation, thromboxane A2 mimetic U46619 was used to pre-contract mouse aortic rings (+/- PVAT). Increasing doses of AMPK activators AICAR (0.1 – 5mM) or A769662 (1-500µM) were added to induce relaxation. Relaxation was measured using a myograph.

**Quantitative immunoblotting and densitometry**

Mouse aorta and PVAT were pulverised and lysed in the presence of phosphatase and protease inhibitors. Total protein assays were performed and samples were prepared for SDS-PAGE and Western blotting. To determine basal or activated AMPK, antibodies for phosphorylated AMPK and the downstream target of activated AMPK, phosphorylated Acetyl CoA Carboxylase were analysed.

**Histology**

Mouse aortae were fixed in neutral-buffered formalin, embedded in paraffin and 4-6µm sections cut on a rotary microtome. Slides were stained with haematoxylin and eosin and morphological analysis was performed with a light microscope.

**Genotyping from mouse ear notches**

Mouse ear notches were processed by PCR using DNAreleasy solution (Anachem), PCR mastermix and forward and reverse primers to wt and ko AMPK DNA. Samples were loaded on to a gel of 2% agarose (with ethidium bromide) and ran at 100V in TAE buffer for 20 minutes. Gels were analysed under UV light and photographed.

3.6 Results: Summarise key findings (300 words max). Please include any relevant tables or images as an appendix to this report:

**Mast Cell Staining**

Positive staining highlighting the presence of mast cells was found in WT and $Kit^{Wsh/Wsh}$ with local mast cell reconstitution samples. No mast cells were seen in $Kit^{Wsh/Wsh}$ or $Kit^{Wsh/Wsh}$ with systemic mast cell reconstitution (Appendix A).

**Small vessel wire myography**

The presence of PVAT in control mice increased AMPK-induced aortic relaxation in comparison to non-PVAT controls. This pro-relaxant effect was not observed in the aortae from AMPK KO mice; where there was little difference in relaxation with or without PVAT (Appendix B).

**Quantitative immunoblotting and densitometry**

The western blot (Appendix C) confirmed the absence of AMPKα1 in the AMPKα1 KO mouse. Levels of AMPKα2 have remained constant and total AMPK (α1+α2) is reduced. Acetyl coA carboxylase (ACC) is also reduced.

Tissue treated with the drug AICAR shows more phosphorylation of AMPK and ACC than untreated tissue (Appendix D) – indicating AMPK activation.

**Histology**

Haematoxylin and eosin staining of a section of control mouse aorta showed an absence of plaques (Appendix E). Time constraints meant that this was the only stain produced by the student, however previous work in the lab has confirmed the presence of plaques in ApoE KO mouse aorta.

**Genotyping from mouse ear notches**

This allowed distinction between control, KO, and heterozygous colonies of mice (Appendix F).

3.7 Discussion (500 words max):

**Mast cells and vein graft disease**

The data obtained is consistent with the existing work of the study. The photomicrographs may have been improved by using a nuclear counterstain. Although

this was attempted, the staining was non-specific. Perhaps if there was more time, alternative counterstains could have been experimented with.

**The impact of PVAT on AMPK pathways and VSM relaxation in health and disease**

The results suggest that PVAT's pro-relaxant effect is only possible in the presence of AMPK, highlighting the importance of AMPK pathways in the action of PVAT. It was shown that the presence or absence of PVAT in AMPK KO mouse aorta made no difference to percentage relaxation. However, in the control mouse, the presence of PVAT resulted in a larger relaxation. Investigations with AICAR demonstrated that treatment with the drug leads to phosphorylation of AMPK and ACC, a secondary messenger in the AMPK pathway, and thus the activation of the AMPK pathway. This is perhaps evidence for AMPK's role in the treatment of metabolic syndrome based disorders, where the action of PVAT is deranged. Immunohistological studies showed plaques in ApoE KO mouse aorta, but not control mouse aorta, evidence which is consistent with current knowledge that ApoE is required for appropriate fat metabolism. Further research in this field could reveal detailed understandings about the role of PVAT in health, and its detrimental role in disease, leading to new treatments perhaps targeted at PVAT. Given more time, it would have been possible to replicate experiments, and also perform a complete morphological study of all mouse colonies, as opposed to solely control mice.

4. Reflection by the student on the experience and value of the studentship (300 words max):

It was unfortunate that, due to lack of ethical approval, we weren't able to achieve the objectives that we had originally set out to achieve, although this demonstrated to me the unpredictable nature of academia. However, the work that I did do in staining mast cells

contributed to the supplementary information of a research paper. I feel that as an undergraduate second year student, this is a good achievement.

The investigation involved using a histological stain that had not been used by the lab before. This was where I learned the trial and error nature of research. It took several attempts for the stain to work properly, each time altering the protocol slightly. Although this was time consuming and frustrating, achieving a positive end result was very satisfying.

I have had the opportunity to learn and practice many new skills in research methods, including wiring small vessels, dissection and slide preparation. I have been taught the importance of accuracy in the lab, how to handle dangerous substances such as ethidium bromide, and the importance of sterility to avoid cross-contamination of substances, in particular when genotyping mice. I believe these skills will translate into my clinical career, where accuracy and infection control are critical to effective patient care.

The experience has enabled me to develop an interest in the field of research and academia. After thoroughly enjoying working in the lab, I am keen to incorporate research into my medical career. I have made the decision to do an intercalated degree, and have also been researching the academic foundation programme, as well as the possibility of undertaking a PhD later in my career.

5. Dissemination: (note any presentations/publications submitted/planned from the work):

The work on mast cell staining will be submitted to a peer-reviewed journal as part of a larger study in the next 6 weeks:

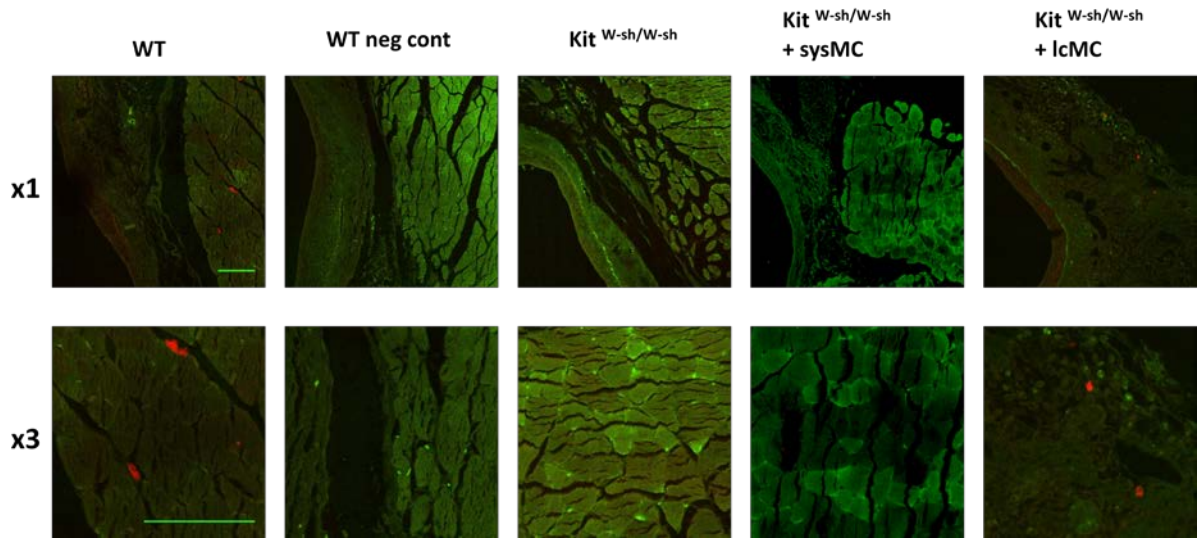
**Perivascular Mast Cells Contribute to Vein Graft Neointimal Formation;** Junxi Wu, Jaclyn Carberry, Neil MacRitchie, Catherine Lawrence, Roger Wadsworth, Pasquale Maffia, Simon Kennedy

6. Signatures: Supervisor  Date 18/9/13

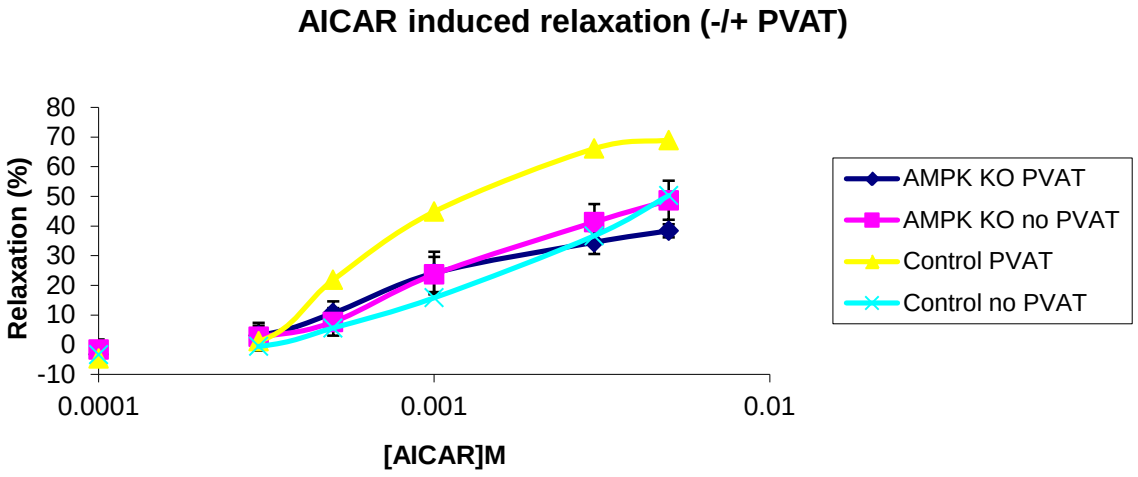
Student Date 18/9/13

## Appendix

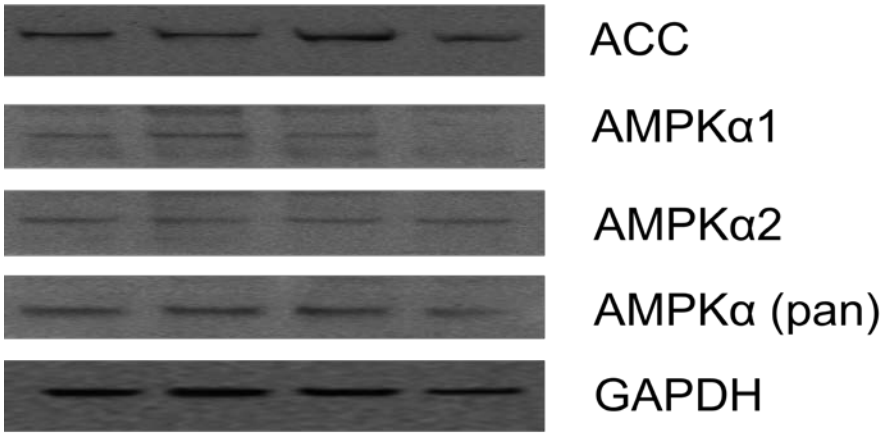
**Appendix A -** Photomicrographs demonstrating expression of mast cells in 4-week-old vein grafts from wild type (WT) mice, $Kit^{W\text{-}sh/W\text{-}sh}$ mice with/without systemic ($Kit^{W\text{-}sh/W\text{-}sh}$ +sysMC) and local mast cell ($Kit^{W\text{-}sh/W\text{-}sh}$ +lcMC) reconstitution identified by Avidin Texas Red staining. Positive staining is red. (Bar = 100 µm).



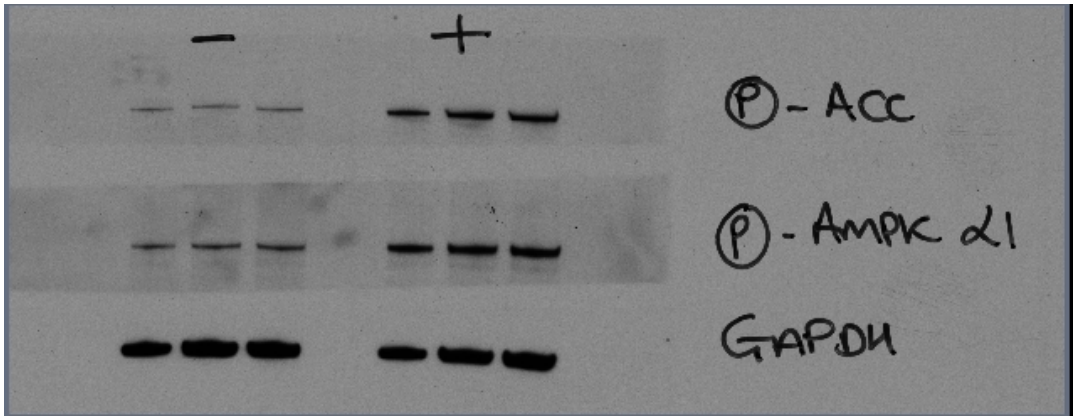

**Appendix B -** Line graph showing percentage relaxation of mouse aorta from AMPK KO mouse and control mouse (-/+ PVAT) when treated with an increasing dose of AICAR.



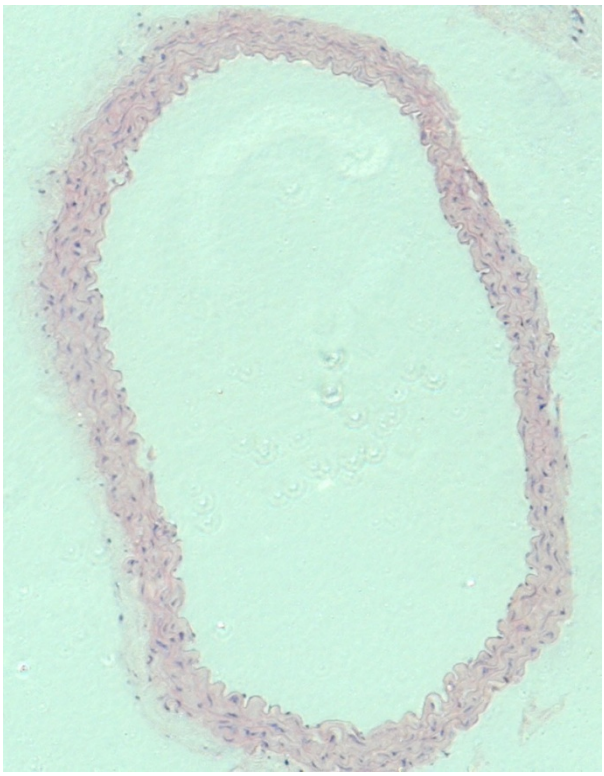
**Appendix C -** Western blot of AMPKα1 KO mouse aorta and PVAT. Secondary antibody detected by means of chemiluminescence.




**Appendix D -** Western blot of control ($AMPK^{+/+}$) mouse aorta and PVAT -/+ treatment with AICAR. Phospho-specific secondary antibodies detected by means of chemiluminesence.



**Appendix E -** Photomicrograph cross section of control mouse aorta. The sample has been stained with haematoxylin and eosin.



**Appendix F -** Agarose casting gel visualised by UV transilumination. Each column represents an individual mouse.

WT

KO