G. NON TECHNICAL SUMMARY (NTS)

Project title: Thrombopoiesis and application to transfusion
Duration of project - years: 5
Duration of project - months: 0

Purpose of the project (as in ASPA Section 5C(3)):
(a) basic research: YES
(b) translational or applied research with one of the following aims:
   (i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their
   effects, in man, animals or plants: YES
   (ii) assessment, detection, regulation or modification of physiological conditions in man, animals or
   plants: NO
   (iii) improvement of the welfare of animals or of the production conditions for animals reared for
   agricultural purposes: NO

(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs
   and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b):
   YES
(d) protection of the natural environment in the interests of the health or welfare of man or animals:
   NO
(e) research aimed at preserving the species of animal subjected to regulated procedures as part of
   the programme of work: NO
(f) higher education or training for the acquisition, maintenance or improvement of vocational skills:
   NO
(g) forensic inquiries: NO

Keywords:
platelets, megakaryocytes, transfusion

Describe the aims and objectives of the project (e.g. the scientific unknowns or
scientific/clinical needs being addressed):

Platelets are small circulatory cells that are essential to prevent bleeding. If platelet numbers are too low, one can transfuse platelets to decrease the risk of bleeding.

The aim of the project is to better understand how platelets are made in the bone marrow and how genetic defect lead to disease of platelets in patients. We will also apply this knowledge to the production of platelets in the laboratory and the testing of these platelets prior to human trials.

What are the potential benefits likely to derive from this project (how science could be advanced
or humans or animals could benefit from the project)?
The sourcing of platelet from blood donors for transfusion to patients has several drawbacks: 1. Sourcing constant supply, 2. donor-derived infections (such as hepatitis, or HIV) and 3. Immune
reaction of the patients against donor blood cells. Producing platelets in vitro would eliminate these 3 issues. The project aims at better understanding how platelet are produced by their bone marrow mother cells (the megakaryocyte) to use this knowledge to produce the cells in the laboratory AND understand how certain proteins that control platelet formation can be targeted by drugs to reduce the platelet count in patients at risk of heart attacks and strokes. In this project we will also test the platelet produced in the laboratory to make sure they function properly after transfusion. This will pave the way for applying to regulators for authorisation to carry out clinical trials with patients.

What types and approximate numbers of animals do you expect to use and over what period of time?:
This project proposes to use mice. Approximately 3000 mice will be used over 5 years.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?:
The animals will be maintained in state of the art facilities, general husbandry will be done by trained staff and experiments carried out by an experienced team. The facilities will also be a “barrier” to infectious agents (to prevent infections particularly in animal with a weak immune system). The majority of the animals maintained under this licence are not expected to show any detectable adverse effects. Most experiments will require the collection of samples after animals are killed either at the end of procedures whilst terminally anaesthetised or otherwise humanely killed. In some instances animal will be administered drugs or cells via an injection in the tail vein or their tummy (or by mouth if appropriate). We may carry out blood test from the tail vein or one of the bigger vein in the leg. These will only cause very limited and short-lived discomfort. In some instances, to be able to carry out the assay described in the project, the animals will need to have their spleen removed under anaesthetic. The effect from this procedure on the animal will be moderate discomfort and will be limited to the post-operative period.

Application of the 3Rs
Replacement:

Where possible, we minimise the use of animals by using platelets or stem cells from human volunteers from which we can grow the platelet mother cells (megakaryocytes). However, the experimental approaches that we can use with human cells are limited. Platelets cannot be stored for more than 1 week and therefore patients with specific disease/mutations affecting platelets would have to be bled numerous times in order to study their platelets in details. The number of stem cells that can be obtained from blood is also very small which means megakaryocytes can only be grown in limited quantities to study how they function. In contrast genetically modified mice will give us the opportunity to study the influence of specific genes on platelets and their production by megakaryocytes by giving access to fresh samples to carry out comprehensive laboratory studies.

Moreover, megakaryocytes and platelets do not act in isolation from other cells. Megakaryocytes need the bone marrow environment to produce platelets and platelets will interact with blood vessel and other blood cells to form a clot. Our analysis of this complex process combines experiments on platelets in isolation from other cells, with experiments in a whole animal setting. This is vital to allow us to analyse gene function in the setting and context of these other cells and to test the quality of the platelets we produce for blood transfusion.

Reduction:

The Home Office, in line with the rest of HMG, has implemented the Government Security Classification (GSC). Details of the GSC can be found at https://www.gov.uk/government/publications/government-security-classifications. Please note that documents and emails you receive may contain specific handling instructions.
Handling Instructions: Contains personal sensitive information, subject to confidentiality requirements under the Data Protection Act. This should only be circulated in accordance with ASPA Guidance and stored in a locked secure location. All government information may be subject to an FOI request and subsequent assessment.
Animal numbers bred for use on this Project will be minimised as far as possible by matching breeding to experimental requirements. Pilot studies and power calculations will be employed to refine the number of animals used.

The methods chosen will generate the greatest amount of data for the fewest animals used. We routinely expect to derive multiple data sets from a single animal, by extensive use of modern approaches that allow us to analyse very small blood samples. This will be complemented by a team approach allowing the analysis of several different samples from one animal.

Refrinement:

Mice are the species of choice for the proposed investigations because they are a good mammalian model with a well-characterised bone marrow and blood vessels that are similar to humans. Studying megakaryocytes and platelets in mice therefore provides valuable information that will further our understanding of human biology and diseases. In particular testing of platelets produced in the laboratory in mice is a well-recognized quality control experiment without which we could not obtain the authorisation to go on to do clinical studies in human patients.

Genetically-altered mouse technologies are becoming increasing sophisticated, where genes can be turned off within specific cells when required, whilst leaving the rest of the animal unaffected. Such genetically altered animals will be used wherever possible in this project and will greatly reduce the risk of adverse effect to the mouse. This is because only the platelets will be genetically altered, whilst all the other cells in the mouse will be normal.