G. NON TECHNICAL SUMMARY (NTS)

Project title: Targeting the immune response in cardiovascular diseases
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: YES
   (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:
Atherosclerosis; restenosis; aneurysm; myocardial infarction; stroke

Describe the aims and objectives of the project (e.g. the scientific unknowns or
scientific/clinical needs being addressed):
Atherosclerosis is caused by the build-up of fatty plaques in the walls of our arteries, and is the cause
of heart attacks and strokes. The accumulation of cholesterol in the arteries leads to subtle chemical
modifications making it recognised by our immune system as a strange 'non-self' material. This leads to
activation of selective white blood cells called lymphocytes, which inflames the vessel. We will try to
identify the immune cells that instruct the lymphocytes to react aggressively against the deposited fatty
material and make the disease worse. The main goal of this part of the research is to determine the
identity of the immune cell subset that initiates activation of our immune system against the deposition
of fatty material in our arteries. Aneurysm is an abnormal local dilatation of an artery. The main
deleterious consequence of aneurysm formation is vessel rupture due to excessive weakening of the
artery wall, which may lead to sudden death. The only treatment involves vessel repair through surgery.
There is currently no approved medication for this disease. My laboratory has recently developed
original in vitro and in vivo experimental models with the aim to unravel the mechanisms of aneurysm
formation and rupture.

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)?:
Our previous research has already led to the initiation of proof-of-concept clinical trials in humans to
test new treatment strategies in patients with heart attack. The present research will lead to a better understanding of how the immune system reacts against the deposition of cholesterol in our arteries and is expected to lead to more effective strategies to combat atherosclerosis and its complications (heart attack and stroke). We also expect that the work will substantially enhance our understanding of aneurysm formation/rupture and will identify critical targets for treatment.

**What types and approximate numbers of animals do you expect to use and over what period of time?:**
Over 5 years period, we will use: Mice: approximately 42850. This amounts to 1.5 mouse/day/researcher. Rats: 280.

**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?:**
We have devised 25 protocols. The majority of animals (70% to 80%) are not expected to show signs of adverse effects that impact materially on their general well-being. No more than 30% of animals are expected to show moderate or severe clinical signs (e.g., piloerection, dehydration, aneurysm rupture). Very rarely the severity of these signs may be such that the humane end points may be reached. Animals are monitored on a regular basis to detect any sign of distress or suffering. Analgesic agents will be administered as required. In the event of complications, or at the end of the experiment, animals will be killed by a schedule 1 method.

**Application of the 3Rs**

**Replacement:**

The number of mice for this project may seem relatively high (although it’s around 1.5 mouse/day/researcher). This is due to the lack of reliable in vitro models of the diseases that we are addressing in this project and to the absolute need to validate any novel disease-relevant targets in vivo using appropriate models.

In addition, many high-ranked peer-reviewed journals require that every experiment is repeated several times to ensure reproducibility.

During the last 2 years, we have developed the use of human induced pluripotent stem cell (iPSC)-derived vascular cells to further reduce and try to replace the use of animals. This has allowed us to replace in some cases one mouse model of cardiovascular disease by iPSC-derived vascular cells (collected and generated from individuals bearing or not the Ap21 risk variant), creating an in vitro model of one aspect of the disease. We will pursue this strategy and try to apply it in other CVD settings.
Reduction:

When designing the experiments, we perform statistical analysis to ensure that we use the minimum number of mice per group that will be informative.

We always aim to maximize the information that can be recovered from a single animal. For example, the same animal may undergo serial imaging in vivo and when killed at the end of the experiment, samples are collected from multiple sites and cavities to assess the effect of candidate gene mis-expression in multiple tissues.

Refinement:

Animals are housed according to the best recommendations in an appropriate and enriched environment. By performing pilot studies and choosing well established protocols based on extensive previous experience, we minimise the unknown effects on the mice and subsequently pain, distress and suffering.

We very frequently monitor animal behaviour and well being to detect any upcoming problem at an early stage.

We have recently refined a model of aneurysm induction using peri-aortic elastase instead of intra-aortic elastase, leading to less invasiveness and much shorter duration of surgery. We will pursue such important efforts to improve our models while maintaining animal distress at the minimal level possible. During the last 5 years of my research, only 1% of animals used under my PPL have shown adverse effects.

In vivo imaging and monitoring of immune cells and atherosclerosis development will be performed on a subset of mice using validated and appropriate methodology. This will allow us to track cell fate in vivo and perform longitudinal studies without the need to kill the animals at each time point of the analyses.