G. NON TECHNICAL SUMMARY (NTS)

Project title: The function of key proteins in T cell signalling and disease
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: **NO**
(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): **NO**
(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:
T cells, Signalling, Immunity, GSK-3, PD-1

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

The main objective of this application is to study the function of glycogen synthase kinase 3 in the immune system. It has previously been shown that treating mice with drugs which inhibit this kinase can suppress tumour growth or viral spread. However, the mechanism behind this is unknown. Using inhibitors and gene-deficient mice I plan to investigate this further and identify other proteins which may be involved. It is uncertain how specific the drugs used to inhibit GSK-3 are and the use of gene-deficient mice will aid to confirm this specificity. During this project, we expect to identity other proteins that are up- or down-regulated in response to GSK-3 inhibition and this could lead to improved or alternative treatments.

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 primary benefit of this research would be to obtain a more complete understanding how GSK-3 functions in the immune response. This could further knowledge in the scientific field and result in the

design of new or improved treatments for cancer and viral infections. Many available drugs are not target-specific and can suppress the whole immune system leading to other infections which can be fatal, improving specificity or identifying new proteins which can be targeted specifically is of extreme importance.

What types and approximate numbers of animals do you expect to use and over what period of time?:
Mice provide the best animal model to study immune function that is very similar to the human immune system and provides a system in which genes can be readily manipulated or deactivated. The minimum numbers of animals will be used that will still provide a statistically valid study (a statistician will be consulted when necessary). The use of pilot studies will help to assess animal numbers and how best to design the main study in order to gain maximum information. We expect to use approximately 12,500 mice over 5 years from the 32,140 requested on the project..

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 majority of animals will make a full recovery in most protocols; those used for tumour induction will indeed grow tumours up to 15mm in diameter but are not expected to show signs of adverse effects that impact materially on their general well-being. In rare cases, moderate clinical signs such as weight loss, stary coat, hunched posture and poor appetite may be observed. In the majority of studies, mice will be culled by a schedule 1 method.

Application of the 3Rs
Replacement:

The need for animal models has arisen from extensive previous studies in the lab where the use of cell lines produced contradictory results that could only be resolved or confirmed with the use of an animal model.

Further to this, *in vitro* work has given rise to possible candidate genes as potential anti-cancer/viral drug targets and it is essential to validating these genes *in vivo* and to analyse their function in tumour/viral development. Where possible *in vitro* work using cell lines will be performed and only extended into animal studies where absolutely necessary. The aim of this project is to identify key proteins in disease which will be initially sought in vitro, but the final aim will be to look at possible treatments using these proteins as targets and therefore will mostly result in the use of animal models for therapeutic purposes.

Reduction:

Statistical analysis, including power calculations, will be used to determine the minimum numbers of mice used while ensuring sufficient data is generated to produce meaningful results.

The use of pilot studies will help to assess animal numbers and how best to design the main study in order to gain maximum information.

To maximise the information from a single animal, we will aim to collect tissue samples from multiple body sites and provide other affected tissues to appropriate scientists, so that they do not have to

breed mice specifically for their experiments.

Non-invasive imaging techniques in live animals and analysis of tissue samples collected post-mortem will allow us to maximise data collection during and after experiments and reduce the total number of animals required.

Refinement:

Mice provide the best animal model to study immune function that is very similar to the human immune system and provides a system in which genes can be readily manipulated or deactivated. In addition, most antibodies for studying immune cell function are available for murine immune cells.

This project continues our existing program of research and builds on our previous findings. We will use genetically modified mice to determine which proteins are involved in these diseases. Interventions such as treatment with immunological reagents or drugs will be used to test well-defined hypotheses. Outcomes will be measured by determining the progression of disease in treated animals compared to non-treated. Blood sampling and imaging studies will provide essential information, alongside *in vitro* tests at the end of each study which will be performed using tissue samples.

Animals will be housed in groups with suitable environmental enrichment. They will be checked daily and regularly handled. When the animals are on study, the frequency of handling and checking may be increased to ensure that the animals are not suffering. The animals will have access to food and water. Blood samples may be taken at regular intervals and other samples e.g. tissues at the end of the study. Blood sampling volumes will be kept to the minimum required to obtain information for this study.

When generating transgenic mice in which a harmful phenotype may be displayed, extra care will be taken in monitoring these mice to minimise suffering and where possible inducible constructs will be used, so that the phenotype is only displayed when the gene expression or deletion is induced.

Throughout the protocols a number of optional administration routes have been provided, this is so that the mice may receive the least intrusive method but yet give the optimal effect. I.e. Intranasal infection of some viruses is the least intrusive method and gives rise to optimal levels of infection, however, other viruses require different routes to give optimal levels. The least intrusive methods will be used where possible.