G. NON TECHNICAL SUMMARY (NTS)

Project title: Haematopoietic and leukaemia stem cell regulation
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:
Haematopoiesis; Stem cells; Leukaemia; Lymphoma; Leukaemia stem cells; Novel Therapy

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

1. Identification of the molecular and cellular events involved in the formation,
maintenance, expansion and differentiation of HSPC and characterisation of how these
regulatory events differ between normal and leukaemic stem cells?.
2. Can we generate appropriate mouse models to study critical genes in HSPC
and haematological malignancies to improve our understanding of their pathogenesis?
3. Can we validate critical regulators of leukaemia and lymphoma biology and obtain pre-
clinical information on novel therapeutics targeting these regulators?

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)?:
This programme aims to identify molecular and cellular regulators of stem and progenitor cell fate
choice. Manipulation of this balance is suspected to play a major role in disease development. This
programme also aims to develop relevant models to understand human disease and provide a system
where new therapeutic strategies and compounds could be tested for their ability to reduce disease
burden in patients. We anticipate identifying 3-5 critical regulators of haematopoietic stem cell function.
and blood formation. Characterisation of each model’s relevance to disease requires careful and time-consuming experimentation but it is anticipated that one or two of the models created in this programme will be used to test potential therapeutic strategies for haematological malignancies.

What types and approximate numbers of animals do you expect to use and over what period of time?:
Species: Mouse We expect to use approximately 6,768 animals per annum over 5 years. (i.e. ~33,820 animals during this 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?:
Since this project will explore the role of previously unstudied genes in the blood system, it is likely that disease will develop in some of these animals, including leukaemia or lymphoma development. Animals will be very closely monitored for signs of disease and killed by a schedule 1 method should the clinical signs necessitate intervention. The majority of mice under this licence will show no signs of adverse effects that impact materially on their general health. It is estimated that more than 40% of animals will not exceed a sub-threshold severity category. Approximately, a further 20% may not exceed mild and ~25% may reach a moderate severity category. Rapid and/or unanticipated adverse effects may rarely be such that the some animals (less than 5%) reach a severe category. Over the last 2 years this has been between 2.8 and 3.2%. These animals may develop haematopoietic malignancies causing abdominal distension, weight gain or loss, anaemia or erythrocytosis, laboured respiration, inactivity or inappetence, combined with signs of hunched posture or piloerection. Animals showing any of these clinical signs will be deemed to have reached human end points and will be immediately killed by a schedule 1 method.

Application of the 3Rs
Replacement:
It is currently impossible to study the complex role of the microenvironment of normal and malignant stem and progenitor cells in vitro since the complete set of important factors have not yet been identified. Also, to study stem cell function, the cell must be shown to possess the ability to sustain lifelong blood cell production and this cannot currently be assayed outside the body. Finally, to assess the function of normal and patient derived human stem and progenitor cells, the xenograft model (where human blood cells are transplanted into mice with compromised immune systems to avoid rejection) is currently the only system capable of determining long-term multi-lineage capabilities.

Reduction:
Colony sizes will be carefully managed to ensure that supply matches the demand, and any surplus mice are used for other scientific purposes and tissues shared over multiple experiments. When designing experiments we perform statistical analysis (e.g., power calculations) to ensure that we use the minimum number of mice per group that will be informative. Finally, we will use human cell lines (including patient derived cell lines) of particular mutations (e.g., CREBBP, DNMT3A) to study the biochemistry of the mutations.

Refinement:

The mouse is the most appropriate and most widely used model for studying blood cells and cancer. The techniques are therefore very well established and findings can easily be integrated with other groups’ data. The mouse is also the species in which reliable gene delivery systems are best established. For our studies that involve blood cell transplantation, we have recently introduced a

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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.
recipient mouse model that permits much lower irradiation doses and we have also removed
techniques that are no longer required from our licence.