Home Office

NON-TECHNICAL SUMMARY

Understanding haematopoietic dynamics in health and disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

We aim to understand how the cells responsible to sustain blood production (blood stem cells) are affected by stresses such as infections or leukaemia growth. Because it is not yet possible to maintain and grow blood stem cells in a test tube, we study them directly in the bone marrow, where they reside, with the aim to understand what other cell types interact with them, exchanging what molecular signals.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Despite bone marrow transplantation having been practiced in the clinic for decades, and being the treatment of choice for an increasing number of conditions, availability of blood stem cells is a critical limiting factor for its applicability. Understanding what cells and molecular pathways support blood stem cells in the bone marrow is critical to learn how to grow them in test tube so that more patients can be treated successfully. Moreover, it is now clear that blood stem cells are damaged by stresses such as infections and leukaemia, therefore it is important that we learn how to preserve them or regenerate themselves following stress. This will enable healthy ageing.

What outputs do you think you will see at the end of this project?

By the end of this project we will have significantly increased the understanding of blood stem cells and leukaemia biology, and identified specific cells and molecular pathways that are promising for the development of novel, improved therapeutic approaches for leukaemia and cancer patients, and for survivors of severe infections. Our findings will be published in highly respected journals (always in open access format) and presented at prestigious conferences. All data generated will be accessible to the research community either directly through deposition in existing databases or upon request by collaborators.

Who or what will benefit from these outputs, and how?

Our data will be useful to drive future research led by us, other research groups and industry in collaboration with clinicians and to drive the development of improved therapeutic and preventative approaches for blood diseases. Patients will be the ultimate beneficiaries of our work.

Outputs from our work will immediately benefit the closer research community, and will reach the global research community once they are published. In the medium term (2-10 years) they will underpin future research projects led by me and others, including potentially clinical trials that test novel therapeutic approaches. In the longer term (5-20 years), our findings will impact human kind in terms of improved therapeutic and preventative approaches in the areas of leukaemia, cancer and infection, and will therefore contribute to develop strategies to enable healthy ageing.

How will you look to maximise the outputs of this work?

I strive to publish all our findings, positive and negative. This is becoming more feasible through the increasing availability of open access data repositories and pre-print publications (e.g. Wellcome Open Research, BiorXiv). Moreover, both myself and trainees in my group regularly present our findings at scientific meetings, and engage with the public and, most importantly, with the technology development, technology transfer and intellectual property departments of both the institutions and the funders who support us. This maximises the opportunities for our work to be evaluated and further developed to drive improved therapeutic and preventative approaches.

Species and numbers of animals expected to be used

- Mice: 16,250

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We work with mice because their blood production system is remarkably similar to the human one. Moreover, they are small, and can be genetically altered, allowing us to test several theories about the cells and molecules causing damage to blood stem cells during leukaemia and infection stress. We work with young to mature animals, typically between 4 weeks and 6 months of age at the beginning of our experiments. This is because we model human adult blood production, and in the mouse, it has been shown that by three weeks of age blood production has completely switched from the foetal/developmental type to the adult/steady-state one.

Typically, what will be done to an animal used in your project?

The length of our experiments, both in terms of actual time they take to be completed and of number of procedures performed on an animal, varies greatly depending on the questions asked and the most relevant models used. For example, our infection models develop within 5-7 days, but our leukaemia models develop within 3-12 weeks. Infections are mimicked by injecting specific substances intravenously or intraperitoneally, or by administering malaria parasites intravenously or via the bites of infected mosquitoes. Leukaemia grows following intravenous injection of malignant cells, with or without preparing the mouse with agents such as irradiation. In the vast majority of cases mice will develop either infection of leukaemia, and not both. Only in later stages of this project, once we have learned the specific damages caused by infection, we will let the mice fully recover from infection and we will subsequently administer leukaemia cells to them, asking whether they are now more susceptible to develop leukaemia. Within these models, we treat mice with substances that activate genes or modify cells that regulate blood stem cell function. These could be hormones administered over 2-4 weeks, or chemotherapy leukaemia treatment administered for 5-10 days. To assess the functionality of blood stem cells, donor mice will be culled and blood stem cells harvested and

transplanted into irradiated recipients, mimicking bone marrow/stem cell transplants performed in the clinic. Specific to our programme of work, we will use advanced microscopy to directly observe blood stem cells and support cells interacting within the bone marrow. This is done under anaesthesia, either as a terminal or recovery procedure. Less frequently, longitudinal studies will require multiple, short microscopy sessions to take place over the course of a few days.

What are the expected impacts and/or adverse effects for the animals during your project?

Mice will experience either mild or moderate levels of severity. Moderate levels of severity are expected for all mice undergoing recovery surgery, and for those experiencing multiple mild procedures within a short period of time (for example daily blood sampling or daily injections).

We expect adverse effects to be very rare (<2% of animals). An exhaustive body of evidence demonstrates that stress affects HSC biology, and it is therefore paramount to us that stress, such as that generated by adverse effects, is minimised, or else we would not generate trustworthy data.

Peri-operative analgesia is provided and animals are expected to be fully recovered within 24 hours, most often within 2 hours from the end of surgery/anaesthesia. Very few animals will undergo more than one surgery (for example, two bone marrow aspirates, or one bone marrow aspirate and one imaging window implantation), and the second surgery will only be performed once full recovery from the first one has taken place and it is clear that that did not cause any adverse effects. We are not currently planning to perform any further surgery on splenectomised mice, and should this become necessary we will discuss it and plan it in tight collaboration with NVS and NACWO, and following the same principle of performing a surgery only if the animal has not shown any adverse effects at any point.

Irradiation is not expected to cause any adverse effects to our animals because either they will be culled before these arise or they will receive sufficient numbers of haematopoietic cells to guarantee their survival. In the latter case, mice will be cytopenic for 3-4 weeks, with the nadir at week 3 post-irradiation. While this is an adverse effect, this does not cause observable clinical signs to the mice. The worst-case scenario is failure of the injected cells to reconstitute the mouse, however this is never planned and therefore it is not an expected adverse effect for this project. If further procedures need to take place following irradiation and injection of cells, these take place either before or after the time window when mice could develop adverse effects, or are terminal procedures performed under anaesthesia (eg intravital microscopy).

Leukaemia cells can cause health deterioration which is only ameliorated by chemotherapy treatment. Animals are expected to respond quickly to treatment and clinical signs to resolve within 24 hours, otherwise humane killing is triggered.

Malaria can develop into cerebral complications, which are lethal. Our experiments are all performed before the onset of these complications, and the onset of signs such as reduced motility and hunched posture is carefully monitored (daily inspections) and triggers humane killing.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Expected moderate: about 30% of animals

Expected mild: about 70% of animals

What will happen to animals at the end of this project?

- Used in other projects
- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

It is currently impossible in the laboratory to mimic the complex three-dimensional relationship between normal blood stem cells and other cells in the bone marrow, and therefore completely replace the use of animals.

Mice are the smallest, less-sentient animal model that can be used to perform the studies described here. They are the only species for which there is a wide availability of genetically altered animals, which makes it possible for us to test the role of specific genes within the blood production system and to visualise cells of interest directly within the bone marrow. Finally, the blood production system that we are studying is remarkably similar to the human system.

Which non-animal alternatives did you consider for use in this project?

We continuously strive to replace animals as much as possible, and provide here some examples. We have implemented mathematical modelling to test theories about certain aspects of blood stem cell biology and simulate interventions. This work allows a partial replacement of the use of animals as it allows us to select parameters to be tested experimentally and therefore reduces the number of animals used. We have also been using our findings from animal studies to develop laboratory systems. For example, we reproduced the disease effects of leukaemia on the cells normally supporting blood stem cells by growing both types of cell in the same experimental dish, and we have been studying the interaction with other cells using similar approaches. In many cases these systems rely on cells taken from mice to set them up, but they do allow us to perform further studies without the need to use more animals although it is only made possible in conjunction with our research findings in mice.

Why were they not suitable?

Commercially obtained cells or those grown in the laboratory are not suitable replacements for cells freshly taken from mice because they are too different and any experimental results would not be relevant to our research.

Specialised techniques using cells are increasingly used in our type of research, but are not yet suitable for research involving blood stem cells, however our findings from animal studies will contribute to improving them.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We use statistical modelling to estimate the ideal number of mice we need in order to achieve meaningful scientific results.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

When statistical modelling indicates that numbers needed are high (for example more than 15 or 20 mice), we use preliminary pilot studies with smaller numbers of mice. Depending on the results, we either abandon that experiment if it is shown not to work or adjust, and often reduce, the number of animals needed if the pilot results are promising.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We breed mice that can have more than one particular trait which minimises the number of animals that we need to use.

By using the same control (untreated) animals and by increasing the breadth of analyses conducted on cells and tissues from each animal, which gives us maximum data per animal, we have in the past been able to use smaller numbers of animals than initially expected. We expect this trend in reduction of numbers to continue in future.

Careful planning of microscopy allows us to follow events as they develop within the same mouse, avoiding issues due to mouse-to-mouse variation and therefore reducing the total number of animals that need to undergo the procedure. Moreover, careful refinement of mouse monitoring and surgery/anaesthesia reduces animal death and ultimately the number of mice required to complete data collection.

In addition, we maximise our breeding efficiency by minimising the number of pairs required through careful crosses and monitoring of all breeding animals.

Allowing malaria parasites through their whole life cycle including in both mice and mosquitoes maintains parasites stocks more consistently, reducing variability and the number of mice required to complete data collection.

The use of state-of-the-art hormone administration protocols increases the efficiency of embryo production, reducing the number of females needed to generate the embryos required.

Finally, as multiple personal licence holders work under this licence, experiments are coordinated so that tissues can be shared as much as possible.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The experimental models of leukaemia and infection used in this project have been selected to minimise pain, suffering and distress to the animals, while providing scientifically relevant data. No lasting harm is experienced by any of our animals.

Of note, the use of naturally occurring infectious agents is a refinement as it eliminates the question whether the observations made following administration of large amounts of specific molecules are relevant to real-life scenarios. Still regarding the infection models we use, and in particular malaria infection, opting for infected blood inoculation rather than insect bites whenever compatible with the specific scientific question addressed reduces the suffering of animals as it does not require anaesthesia and it shortens the overall duration of the infection.

The use of sophisticated genetically altered animals where mutations are restricted to specific cells of interest instead of affecting the whole organism drastically reduces the emergence of adverse effects due to the genetic background of the animals. For this reason, our breeding protocol leads to only mild levels of severity experienced by the animals.

The use of sterile males whenever possible reduces the need for vasectomy and therefore avoids using surgery to achieve the same output.

Finally, throughout our experiments, mice are carefully monitored at a minimum through daily inspections, which increases to twice daily at times when adverse effects are expected (for example at late stages of leukaemia development, or the peak of malaria infection). Monitoring may be undertaken continuously in some situations, for example for the few hours following recovery surgery, to ensure recovery is smooth and prompt.

Why can’t you use animals that are less sentient?

The mouse is the ideal model organism to study blood stem cell systems as it is most similar to the human. Less sentient animals such as zebrafish and the fruit fly can be used to ask very specific questions, but their blood stem cell system is too different from the human to address our research questions.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Our past experience has brought to our attention specific adverse effects that can be monitored and minimised. For example, we refined our breeding protocol (P1) to identify certain genetically altered animals by eye inspection using fluorescent goggles whenever possible. As a result, we have been able to eliminate tail clipping as a procedure.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

LASA guidelines for the administration of substances are followed at all times. Moreover, NACWOs and NVSs bring to our attention relevant publications describing practices that we can implement. For example, as a result of such interactions we have implemented the body conditioning score system to ease the identification of human end points in all our experiments.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Because this is a follow-on project licence (PPL), the majority of the protocols have been revised and refined from the previous PPL I held.

All protocols undergo constant refinement as new and improved approaches are developed that minimise mouse discomfort and allow better welfare monitoring. Continuous collaboration with local Named Animal Care and Welfare Officers (NACWOs) and NVS ensures that our work is constantly refined and always up to date with any 3Rs advancements. In particular scheduled PIL re-trainings have been proving an excellent approach to ensure implementation of any 3Rs advances.