Home Office

NON-TECHNICAL SUMMARY

Dissecting the intrinsic and extrinsic mechanisms regulating normal and leukemic stem cells

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
- (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 following aims mentioned in paragraph (b)

Key words

Blood Stem Cell, Leukaemia, Microenvironment, Bone marrow niche, Therapy

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

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?

The aim of this project is to study the normal development of blood stem cells and understand how and which alterations might alter this normal development and induce leukaemia. We will also investigate how normal and leukemic cells respond to specific microenvironment factors and investigate whether modulating specific factors could impede the growth of leukaemia. Lastly, we aim to translate our basic laboratory research into clinical benefit.

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?

Blood stem cell transplantation is a life-saving therapy, employed to reconstitute cancer patients' blood and immune systems. Furthermore, blood stem cell could be engineered to correct genetic diseases or fight infectious agents. Nevertheless, shortage of blood stem cell donors still limits their used. Understanding what mechanisms regulate blood stem cells self-renewal and their fate decision should provide new tools to generate, and expand blood stem cells and thus exploit their full therapeutic potential. Furthermore, dysregulation of blood stem cells via acquisition of genetic mutations are at the origin of leukemia development. Understanding how leukaemia initiates, propagates and competes with the normal blood compartment will allow us to develop new therapeutic tools.

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

At the end of the project we anticipate to:

- Have a better understanding of the components of the stem cell niche using visualisation techniques and functional study to elucidate which component of the stroma is/are essential for the maintenance of normal and leukaemic stem cells.

- Unravel how microenvironment cells/factors influence human normal haematopoietic stem cell (HSC) fate decision and thymopoiesis.

- Decipher how pre-malignant and malignant cells outcompete overtime and/or under different stresses (such as inflammation, chemotherapy, radiation, infection, mobilizing agents).

- Provide new insight into the regulation of human haematopoietic stem cell (HSC) during ontogeny and ageing.

We will disseminate our results in conferences and publications as well as in public engagement events.

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

As we are working directly on human haematopoietic stem cells and leukaemia, data generated should provide:

- new therapeutic avenues to target malignant stem cells while preserving the normal stem cell compartment.

- new ways to expand the stem cell pool providing a new source for cell therapy like BM transplantation.

- new ways to harness human T cells development.

- novel model to investigate the effect of leukaemia in immune cell function and develop new immunotherapy strategies.

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

- Thanks to a constant discussion with the translational team at the Institute, we are able to patent our works, and via direct communications with big pharma and biotechs are in a good position to translate our work.

- Similarly, thanks to good connection with clinicians we can translate some of our work to the clinics.

- Via collaborations (internally and externally), we are and have been able to extend our work (for example: investigating the role of neutrophils in leukemia; developing human thymus).

- Publication of method papers or reviews.

- Participating in public engagement events.

Species and numbers of animals expected to be used

- Mice: 25,000

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.

Mice are chosen because they have a short mammalian life span, are easy to maintain, and have similar physiological, anatomical, and genetic traits to humans. They are also the only form of mammal

in which transgene technology works well and immunodeficiency models have been established. Furthermore, previous works from our group and others have shown that immunodeficient mice allow human blood system development, both normal and leukaemic. Thus, it is the model of choice to study human normal and leukaemic stem cells.

Indeed, the use of immunodeficient mice has been instrumental in the demonstration of the cancer stem cells in AML as well as to our understanding of the heterogeneity of the normal HSC compartment.

New immunodeficient mice have been used over the years, which provide a better environment for the development of human normal and LSC (like NSG: Il2Rgamma null, NSG$^{W41/W41}$ mice). We will continue to test new models has they become available.

We usually perform our experiment on adult mice, but in some cases, the used of newborn, or juvenile mice to evaluate HSC function during ontogeny might be more appropriate.

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

In a typical scenario, animals might be subjected to different types of conditioning (irradiation, busulfan etc.), followed by the adoptive transfer of cells via usually intravenous (IV) or intra-bone (IBM) injection. Following the adoptive transfer, mice might also be subjected to imaging under anaesthesia, and they might have blood and/or bone aspiration taken for analysis to test level of engraftment. In some cases, in addition, usually 6 to 12 weeks after adoptive transfer, mice might be subjected to injection of anti-cancer drugs. In other cases, animals might receive different types of challenges (infection, inflammation stress,etc). All mice will finally be killed typically about 12-36 weeks after adoptive transfer, which may involve perfusion or exsanguination under terminal anaesthesia. Lastly, some mice might be implanted with scaffolds either subcutaneously or in limited number of cases, under the kidney capsules. For subcutaneous implantation, an imaging window chamber might be placed to image cells proliferation, and mobility overtime. In all cases, we will use non-invasive technique to follow the development of both normal and leukaemic cells engraftment.

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

In most cases, animals may experience pain and weight loss for a short period of time (mild to moderate severity). Mice transplanted with leukaemia might become sick via dissemination of leukemic cells in other tissues. Mice will be monitored daily and at any signs of sickness will be culled. Thus, maximum severity should not exceed a moderate level.

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)?

Immuno-compromised mice are usually more susceptible to the procedures and thus might reach the moderate severity limit of the protocols.

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

- 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?

The gold standard assay to study and prove that the cells studied have normal HSC or leukaemic stem cells activity is to transplant them in vivo into an immuno-deficient mouse model. Thus, the use of animals is currently unavoidable as many facets of stem cell and leukaemia biology are only apparent in the context of the complex in vivo systems in which these cells and diseases naturally occur.

Mice were chosen because they are the only form of mammal in which transgene technology works well and immunodeficiency models have been established. Furthermore, previous work from our group and others have shown that immunodeficient mice allow the development of human blood system both normal and leukaemic. Thus, it is the model of choice to study human normal and leukaemic stem cells (LSCs).

Indeed, the use of immunodeficient mice has been instrumental in the demonstration of the cancer stem cells in AML (Bonnet et al, Nat Med. 3: 730, 1997) as well as improving our understanding of the complexity of the normal HSC compartment (Afonso et al. Cell Stem Cell, 2013). We also recently developed a humanised 3D ossicles which allow us to better mimic the human bone marrow niche (Abarrategi et al, JCI, 2017).

New transgenic immunodeficient mice are being developed that provide a better environment for the development of human normal and LSC (like NSG: Il2Rgamma null, NSGckit$^{W41/W41}$, NSG-S). We will continue to test new models has they become available.

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

Over the years, we have developed an ex vivo co-culture system that allows the maintenance of some normal and leukaemic stem cells. We are also in the process of developing a new 3D bioengineered bone marrow system that mimic the human bone marrow niche ex vivo and thus should be provided an even better environment for human normal and leukaemic stem cells.

Why were they not suitable?

Despite continuously improving our ex vivo culture system, the use of animals is currently unavoidable as many facets of stem cell and leukaemia biology are only apparent in the context of the complex in vivo systems in which these cells and diseases naturally occur.

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?

In the design of all our mouse experiments, we strive to use the minimum number of animals that is commensurate with obtaining a robust and reliable result. We will apply optimal experimental designs and statistical analysis as key means of achieving reduction. We will use power calculations to minimise the number of animals used in each experiment. For the design of most of the quantitative experiments, sample sizes will be set using power analysis, generally using a significance level of 5%, a power of 80%, and at least practicable difference between groups of 20%. We will use statistical power analysis under advice from our bioinformatics and statistics core service to determine the appropriate minimum number of animals per study required to gain significant data output.

In general, to investigate the effect of specific over-expression or down-regulation of a target gene, a group of 4 to 6 mice should be enough (control and experimental group). For evaluating the effect of anti-cancer drugs against leukaemia, it is generally more difficult to predict a minimal number based on the heterogeneity in the time to develop leukemia (6 to 18 weeks). Still our past experiences indicate that a group size of around 10-12 mice should be appropriate.

Otherwise, we will use the minimum number of animals to provide an adequate description, generally based on previous experience (our own or from the literature). We will use a pilot experiment, for example, for preliminary studies, e.g. to optimise the dose of cells, no more than 3 animals per group will be used when possible.

Experiments will be carefully planned to maximise the information obtained per animal and limit the subsequent use of additional animals. For example, organs including bone marrow, blood cells and spleens will be stored and used for multiple experimental purposes. We will also make use of non-invasive imaging to maximise the information obtained per animal.

Collaboration with research colleagues will help to gain maximum data output and reduced redundant breeding/experimentation.

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

Longitudinal measurements, in particular non-invasive ones, such as weight loss, and non-invasive imaging, allow gaining a wealth of information on disease course over time with a minimum number of mice used. Cryopreservation of tissues and cells is routine at the establishment and will ensure that the minimum number of mice is bred and measures are in place to maximise efficiency of breeding schemes with a minimum surplus. Reporting will be based on ARRIVE guidelines. Imaging technology will allow following a cohort of mice over time rather than setting up several experimental groups to allow kinetic analysis, e.g. investigate leukemia invasion overtime or the effect of anti-cancer drugs. We will remain alert to any advances, which will enable the replacement of animals.

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

The efficiency of animal usage is maximised in consultation with colony managers and animal technicians, by careful control of breeding to meet research needs with respect to numbers, phenotypic uniformity and health. This has been greatly facilitated by a mouse database in which every breeding pair and every mouse born are recorded and through which we can readily monitor the numbers of mice we hold.

Where possible, lines will be maintained in a homozygous state, thereby obviating offspring with undesirable genotypes. Littermates genotyped as heterozygous or wild type from the breeding protocol will be used as appropriate age and gender matched controls. This allows optimal use of mouse numbers generated and is the best scientific practice for the study of genetic alterations.

For experiments on development and progression of a leukaemic disease (leukaemogenesis) in response to anti-cancer drugs, we will, whenever possible, make use of bioluminescence imaging to follow a cohort of mice over time, which will substantially reduce the number of mice involved. We will also make used of our new 3D ossicles model, which allows us to implant up to 6 ossicles/mouse, allowing us to reduce the number of mice needed /experiment, as each scaffold could be analysed separately. Lastly, this work program will make optimal use of several tissues, fluids and cell types per individual mouse. This highly integrative approach will maximise the information obtained from the minimum resource.

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.

Mice are chosen because they are the most frequently used mammalian model system to study biology of stem cells and cancer. Other advantages of the mouse model include the availability of antibodies to identify and purify different classes of haematopoietic stem cells and mature blood cells and the availability of in vitro (proliferation, colony-forming cell assays) and in vivo (repopulation experiments and serial transplantations) functional assays.

Previous work from our group and others showed that immunodeficient mice allow human blood system development both normal and leukaemic. This makes the xenotransplantation model a perfect choice to study human normal and leukaemic stem cells.

As most of our work will involve the use of highly immunodeficient mice, we will breed these mice under specific isolators and maintain the experimental mice in IVCs (individually ventilated cages) under a barrier environment, to avoid infections. Furthermore, based on our previous experience working with

these mice, when conditioning of the mice is necessary (use of for example sublethal irradation, busulfan etc.) we will be using acidified water and a course of 10 days antibiotic treatment post-conditioning.

In our experiments, we will set clear humane endpoints and, as part of good laboratory practice, write an experimental protocol for each experiment, which will include details of possible adverse effects. All staffs involved in the experiment will have access to these protocols. In addition, when considering which route of administration of substances to employ, we will strive to use the least invasive route while maintaining direct control of dose.

Administration of substances and cells: the route to administer a substance or cells should be such as to achieve “best practice”, that is, to minimize or avoid adverse effects, while minimizing the number of animals used, and maximizing the quality and applicability of results (Morton DB, et al 2001). For that reason, we propose in this project licence, a variety of routes of administration of substances and cells to achieve the scientific objectives, while minimizing the waste of animals' lives. Although in the most cases, we will primarily use standard routes of administration such as intravenous or intraperitoneal injections, the active concentration, volume, stability, and toxicity of a particular substance may require its administration through a non-standard route (like gavage of anti-cancer drugs). Similarly to bypass potential complications arising from failure of the transferred cells to establish in host organs, we will directly inject cells into host organs such as long bones, foetal liver (in case of newborn injection) or implant our scaffold in the kidney capsule (for best vascularisation).

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

Juvenile or adult mice are chosen to study human adult stem cell development as well as leukaemia as they provide at this age an equivalent adult bone marrow microenvironment. Similarly, neonates will be used for studying foetal human hSC development.

Previous work from our group and others showed that juvenile/adult immunodeficient mice allow human blood system development both normal and leukaemic.This makes the xenotransplantation model a perfect choice to study human normal and leukaemic stem cells.

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

We will work closely with the veterinary staff to ensure that we are always refining our protocols to minimize harms for the animals used in our research.

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

We will stay up to date with the best practice guidelines developed by the National Centre for the Replacement, Refinement, & Reduction of Animals in Research, and the scientific literature for estimation of sample sizes based on power calculations.

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

We keep up with the latest developments in the field by reading the relevant literature and we have ongoing discussions of 3R measures in the institute which ensures we are always up to date.