Home Office

NON-TECHNICAL SUMMARY

Extrinsic regulation of haematopoietic stem and progenitor cells in health, disease and as a therapeutic target

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

Key words

Blood stem cells, Leukaemia, Microenvironment, Therapy, Transplantation

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, 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 overall aim of our Project is to better understand how blood and immune defence cells are produced to improve the outcome of blood stem cell transplantations and the treatment of diseases of the blood system.

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?

To advance our knowledge on how blood cell production is controlled by its surrounding environment and to overcome key current limitations in the treatment of cancer, like the resistance of cancer cells to therapies or the occurrence of undesired effects of the treatments, such as a reaction of the cells transplanted to fight cancer against the own body (graft vs host disease, or GvHD).

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

The expected benefits of our programme of work are: a) To broaden our understanding of how blood-forming cells crosstalk to their surrounding tissue in health and disease; b) Dissect the exact role of several cell types regulating blood stem cells; c) Use the new information we acquire to devise new therapies for blood disorders; and d) Explore new or improved therapies for patients who suffer blood disorders or in need of a blood stem cell transplantation.

It is expected that intellectual property emerging from this project will support at least two planned patent applications for a biomarker kit and for therapeutic compounds. These data and intellectual property will support future funding applications and clinical trials to improve the outcome of blood stem cell transplantations and the treatment of blood cancers.

Possible long-term benefits for patients are evidenced by the fact that the blood disorders studied represent a significant clinical need. Acute myeloid leukaemia (AML) is an aggressive blood cancer with poor overall survival (<30% long-term). Curative treatment is arduous for the patient, expensive and requires prolonged hospital stays, representing a significant cost to the NHS and a social burden on the UK. Stem cell transplantation, where stem cells are provided by compatible donors, provides a powerful approach, but is also costly and associated with significant toxicity. Taking these factors into account, AML remains a largely unmet medical need. The myeloproliferative neoplasms (MPNs) are chronic diseases with no cure currently available, except for blood stem cell transplantation, which is only recommended in a minority of patients. MPNs significantly reduce life quality and are costly given their chronic nature and associated complications, such as cardiovascular complications (blood clots or

bleeding) or bone marrow scarring impeding normal blood cell production and associated with high risk of developing AML.

Our own work and cumulative evidence indicate that the surrounding tissue (microenvironment) of blood stem cells promotes MPN and AML development, and protects AML cells from current therapies. However, the underlying reasons (which could offer new therapies) are only partially understood. These diseases are more frequent with advanced age, but whether changes in these tissues during ageing explain their increased incidence in older patients, remains unknown. Our data generated in the previous Project has provided relevant clues. Translation of this research into patients fructified in clinical studies investigating the possible redeployment of available drugs for MPN treatment. The research proposed has the translational goal of increasing the number of patients that could potentially benefit from these therapies. The data generated by our programme of work will contribute to the knowledge related to the blood stem cell environment and potentially offer new therapeutic targets.

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

The short-term benefits will be increased knowledge of 1) the mechanism controlling the division of normal and leukaemic stem cells; 2) how neural-related signals control the division and activation of blood stem cells and immune cells and 3) why specific abnormal cell-cell interactions occur and why these can lead to blood diseases. The long-term benefits could comprise new methods to prevent severe outcome of blood stem cell transplantation, and/or new therapies (alone or in combination) to improve these outcomes or the treatment of blood cancers.

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

All advancements in our work are immediately circulated within our group on a weekly basis, typically during weekly lab meetings, and on a quarterly basis within the Department. We have several collaborators who are frequently updated with our results, depending on their relevance to their research. Additionally, we present talks and posters in international conferences and publish all our findings in peer-reviewed, open-access journals so that everyone can retrieve this information. Since the peer-review process usually takes some necessary time, for timely report of our findings we will continue to deposit our research timely in public repositories, such as BioRxiv and ResearchSquare. We will disseminate also unsuccessful approaches for the sake of transparency and increased level of information via open access, including platforms such as F1000Research, where we’ve published before. We collaborate with the pharmaceutical industry to access their expertise, drugs and animal models that can be used to devise therapeutic approaches. Newly devised therapies will continue to be tested by collaborator clinicians through clinical studies.

Species and numbers of animals expected to be used

- Mice: 19,050

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.

Mouse models have been used extensively in blood research because blood production is best established in the mouse. Our group investigates the complex crosstalk between stem cells and their microenvironment, comprised by a multitude of different cell types but also structures (bones, vessels, nerves). It is currently not possible to model this complex microenvironment in the lab.

Our studies will be mostly focused on postnatal stages of blood cell production. Our group is focused in studying blood cancers that occur in adult individuals. Therefore, we will focus our animal studies on adult mice. However, sometimes we need to build the disease model through an early procedure, before the experiment takes place; when that is the case, we will include neonate and juvenile life stages in our studies.

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

We use mice as most of the desired genetic modifications have already been established in genetically-modified mice and our results are immediately comparable with published literature. When using genetically-modified mice (GAA, Genetically Altered Animals), we will try to use models where we can control the manifestation of disease or harmful observable features (phenotypes) to reduce the time and grade of such phenotypes, which we will closely monitor, score and record in spreadsheets. Most of the mice we use are not expected to present any adverse effects. The vast majority of our procedures will be minimally invasive and are not expected to produce long-lasting harm.

We will mainly use the genetically-modified (GAA) mice bred in house. Occasionally, we will obtain wild type (WT) C57BL/6 or BALB/c mice or GAA mice from external sources. GAA mice comprise disease models for blood cancers, such as acute myeloid leukaemia (AML), myelofibrosis, myeloproliferative neoplasms (MPN), juvenile myelomonocytic leukaemia (JMML), as well as patient-derived xenotransplant (PDX, an animal model of cancer where human blood cells form a patient's tumour or a control donor are implanted into a mouse) and Graft versus Host Disease (GvHD, a systemic disorder that occurs when the transplanted cells attack the recipient's body cells) models. However, in most models the disease induction can be experimentally controlled and surrogate markers (e.g. blood counts) are available in some cases to avoid the development of harmful phenotypes.

In our leukaemia experiments, we try to minimise the time that the animal is sick and during that time, we try to reduce the burden of the disease using therapeutic agents, in line with our research questions.

Mice with a deficient immune system (immunodeficient mice) may be used as recipients for human blood-forming or leukaemia cells. In order to study blood cancers in their full complexity and develop effective therapeutic approaches for tackling them, it is necessary to establish in vivo models. This requires the use of immunodeficient mice, which are capable of sustaining human normal and malignant blood production following transplantation. A minority of the immunodeficient mice (NOD scid gamma, or NSG mice) and derived strains animals in this proposal (up to 25%) will develop an overt cancer. The mice usually develop the disease 2-5 weeks after induction; however, the development of

blood cancers cannot be accurately predicted with complete certainty, as it does not always progress in a stepwise fashion. Therefore, after induction, these mice will be closely monitored by regular checks and serial blood counts, which will be recorded in the database and scoresheets. Blood counts allow us to detect and control the development of leukaemia and establish surrogate endpoints before adverse symptoms appear, and therefore we will be able to treat or kill the mouse before overt leukaemia develops.

One of our most frequent procedures is the transplantation of blood-forming stem cells (HSCs), which is the gold standard method to determine their stem cell function. Transplants usually require an irradiation step. Irradiating the animals is the only way to replace the haematopoietic system of a mouse with donor-derived cells. There is no alternative that can generate reproducible results, and efforts have been set to reduce the suffering caused to the animals. By using a split-dose protocol, providing dietary supplements, acidified water and prophylactic antibiotics (after consultation with the named veterinary surgeon), and enrichment to the cages, mouse welfare is significantly improved and re-established after irradiation.

On occasions, we will use treadmill exercise an alternative established and non-invasive model to stimulate the activity of the nervous (avoiding the requirement of drugs). Only moderate exercise will be used, never reaching exhaustion.

In the rare case of invasive procedures with a larger impact, such as terminal bleeds and a subset of surgeries, the animals will be anaesthetised prior to the procedure but will not be recovered after that, and therefore they will not experience any pain.

These studies require the administration of substances (either cell populations in suspension or drugs). When administering a compound, we will use our previous experience and will study the available literature to predict possible side effects and determine the most appropriate dose and administration route. When sufficient information is not available for a specific procedure plan, a pilot study with a reduced number of animals will be designed to assess potential toxicity and efficacy, starting with a low dose through the route with lesser side effects. In pilot studies, or if a substance is being administered to a particular new strain for which side effects may be enhanced, increased health checks will take place for closer follow-up. These measurements will allow to detect an unnecessary harm and to improve endpoints and reduce possible phenotypes.

Rarely, we might need to perform a surgery for an experiment. For example, some experiments require implantation of a subcutaneous minipump or pellet, particularly when a given substance needs to be systemically administered for a long period of time and this approach is considered to be the most appropriate for the animal’s welfare. Surgery is also needed to adhere an imaging window to the surface of the mouse skull to be able to image live cells through fluorescent microscopy in the bone marrow. This imaging modality is the only feasible way to study how live HSCs interact with their microenvironment. These experiments are only performed in a highly selected number of strains and mice by highly skilled and experienced researchers, who have optimised the technique to enable a rapid and unremarkable recovery of the animals. These procedures will take place in a room free of pathogens (SPF) in accordance with the Home Office Minimum Standards for Aseptic Surgery and the Laboratory Animal Science Association (LASA) Guiding Principles for Preparing for and Undertaking Aseptic Surgeries, to ensure the least possible distress and lasting harm to the animals.

In a typical experiment, a mouse around 8-week old, commercially purchased or breed in-house, will be added to an experimental group. Any incoming mouse will be allowed to acclimatise to the animal facilities. The mouse will receive a split-dose of irradiation followed by an administration of cells in suspension through a vein. After up to two weeks of recurrent health-checks to prevent the adverse health effects, the mouse will receive drugs (through oral -food pellets- or injection). The mouse will be periodically monitored through small blood samples taken and a variety of blood cell counts will be recorded in the database and scoresheets. These will allow us to detect the early-stages of haematopoietic recovery and/or the initial steps and progression of blood cancer. In blood cancer (leukaemia) models, blood counts will normally reach the necessary threshold 2 to 8 weeks later, when the mouse will be administered the treatment, mainly through the injection of therapy drugs. Upon treatment completion of treatment or disease re-appearance (detectable by the blood counts), the mouse will be killed for tissue analysis.

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

In order to study blood cancers, we need to induce these diseases in mouse models. However, disease manifests by altered blood counts, which normally precede harmful phenotypes and allow to avoid animal suffering. One exception is the mouse model for GvHD, where transplanted cells react against the recipient body and (like the human disease) affects multiple organs leading to a range of symptoms up to moderate threshold (i.e., diarrhoea, weight loss).

Most of the procedures carried out in this project are not expected to have an impact greater than mild and transient in mice. The main adverse effect expected in our studies is a transient weight loss. However, the overall impact of this weight loss is minimal due to a close follow-up and provision of dietary supplements, which normally leads to weight recovery after the procedure.

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)?

Based on our experience with similar procedures, the expected severities will be:

Species	Severity	Proportion of animals
Mice	Sub-threshold	20%
Mice	Mild	65%
Mice	Moderate	15%
Mice	Severe	N/A

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

- Killed
- Used in other projects
- Kept alive

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?

Mouse models have been used extensively in blood research and blood cell production is best known in the mouse. Transplantation of blood stem cells is the gold standard method to determine their function, like the capacity of the injected cells to regenerate the blood system of the recipient and give rise to all the different types of blood cells. Moreover, the blood flow, which is key to the study of endothelial cells, as well as the electric pulses originating from the central or peripheral nervous system, are difficult to mimic in the lab. Additionally, our group investigates the complex interactions between stem cells and their home, comprised by a multitude of different cell types but also complex structures (bones, vessels, nerves). It is currently not possible to model these complex interactions in the lab.

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

We will be implementing non-animal alternatives in this project, as we have already been doing for the past years. We will be using alternative approaches (without involving animal experimentation), when possible. The most frequent and successful alternatives used are optimised culture protocols, such as co-cultures of different cell types. We have improved methods to maintain normal and malignant blood-forming cells cultured together with another type of stem cell found inside our bones (mesenchymal stem cell, which gives rise to bone, fat and cartilage).

In the upcoming studies we aim to further replace animal use through the use of bioengineered models (functionalised scaffolds that can maintain different cell types in culture). We are currently starting a collaboration which will allow us to study some aspects of the bone marrow environment in the lab, using an upgraded version of material and solution that allow us to maintain different types of blood-forming cells in culture, in conditions similar to real life. This sort of Replacement will allow us to reduce the number of animals in some studies.

Why were they not suitable?

Laboratory models cannot replicate the complex signals and interactions that take place in the body; therefore, we cannot limit our research to non-animal alternatives. These alternative approaches are only a suitable option when the research is focused on a particular cell type or interaction. In those cases, we have devised culture conditions that can serve as research models in the lab. However, in most cases the validity of the results would still require confirmation in live animals, for translational purposes.

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 have estimated the numbers of animals based on our previous project and experience, the annual returns of procedures and changes in the mouse lines and the research goals needed.

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

We follow the recommendations under the publication “Guidelines for the design and statistical analysis of experiments using laboratory animals”. We calculate the sample size, taking into account specific response variables, the experimental groups to be compared and the minimum biologically relevant differences to be detected between experimental groups.

To reduce the number of animals used, we will try to use as many tissues as possible from each animal and store different samples that can be used for diverse assays. Surplus tissues will also be given to collaborators and other researcher scientists (upon request) to reduce the number of mice needed for research. Further optimisation of the use of tissues from the same mice for multiple assays has allowed us to reduce the number of mice originally forecasted in our previous Project.

The number of animals used in the entire programme of work will be minimised by careful planning and scheduling of breeding and experiments and by using the minimum number of animals to answer the question posed. By performing pilot studies and in-depth research on the scientific literature and by developing alternative methods (such as upgraded culture methods that require less cells and animals) we have been able to reduce the number of mice originally forecasted as necessary, compared with our previous Project.

Among all protocols, the ‘Breeding and maintenance of genetically altered animals’ protocol is the one that requires the largest number of animals, although most of these animals have a continued use in the experimental protocols (since the experiments mainly use animals bred in house). For this reason, concerted efforts optimise the breeding efficiency to avoid a surplus of animals. For this purpose, breeding is kept to the minimum for the maintenance of colonies and researchers plan their experiments with sufficient anticipation to increase the number of breeders, if needed. It is normally required to do multiple (2-3) replicates of each experiment for scientific validation. When possible, studies are repeated on the same animals, and the results can be compared with the previous ones, thereby reducing wastage. Some genetically modified mice can be bred in a pure genetic combination background to avoid surplus of animals. However, in most cases mice with combined genetic background will need to be bred, unavoidably resulting in the generation of more animals. When this is the case, animals are offered to other researchers for their possible use.

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

Colony management is performed daily by colony managers and weekly by the principal investigator (Project Licence holder). Mid- and long-term plans of strains are reviewed on a monthly basis. We proactively stop those colonies that are not planned to be used in the near future, and maintain frozen sperm or embryos for strains that are not available commercially or in public collections.

An active communication system within our group and with other researchers, together with frequently updated databases, allow an efficient management, despite the high number of mice and strains required for this Project. We use an online database with all the current animals and the studies assigned to them (or their availability) and an internal chat group for an agile communication regarding mouse colony management. Breeding is carefully planned by researchers well in advance and the future mouse litters have a study already assigned before being born. Surplus animals, mainly due to their unwanted genetic combinations (which are difficult to predict), are allocated for experiments within our group or to other researchers, when possible. Frequently, lab members can make good use of surplus animals for cell obtention (lab studies) or pilot studies, thereby avoiding the need to obtain additional animals for those purposes. Also, we actively collaborate with other groups to share mouse tissue when possible, to optimise the use of each animal and reduce the overall number of mice used in different projects.

Lastly, we take advantage of pilot studies with a reduced number of mice, which are especially useful when sufficient evidence is not available in the literature, databases, guidelines or from own experience, to assess the procedures and define potential side effects, the smallest drug amount needed to obtain a result, the ideal time points for analysis and the best way to provide the drug. These pilot studies will ensure the best experimental design, and consequently reduce the overall number of mice used for research.

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.

Using mice as a model system has well-established benefits such as: a short breeding time, large litter numbers, short life span, easy genetic modifications, and easily translation to human therapies.

We use mice as most of the desired genetic modifications have already been established in other labs making ours immediately comparable with the published literature. When using genetically-modified mice, we will try to use models whereby we can control manifestation of disease or harmful phenotypes to reduce the time and grade of such phenotypes, which we will closely monitor, score and record in spreadsheets. Most of the mice we use are not expected to present any adverse effects and

the vast majority of our procedures will be minimally invasive and are not expected to produce long-lasting harm.

We will be mainly using the mice we breed, and occasionally we will purchase wild-type mice from commercial sources. Genetically modified mice comprise disease models for blood cancers and GvHD. However, in most models the disease start can be controlled and the blood samples taken frequently tell us when the disease starts so that we can reduce animal suffering by treating or killing the mice at that time.

In our blood cancer animal experiments, we try to minimise the time that the animal is sick and during that time, we try to reduce the burden of the disease using therapies, in line with our research questions. Pain management will include the use of medicated palatable substances for voluntary treatment such as flavoured jelly, paste or milkshake liquid.

Mice with a genetically-modified immune system may be used as recipients for human cells. In order to study blood cancers in their full complexity and develop effective therapies, it is necessary to establish humanised animal models. This requires the use of mice with a defective immune system, which are capable of sustaining human blood cell production after human blood stem cell transplantation.

One of our most frequent procedures is the transplantation of blood stem cells, the gold standard method to investigate these cells. Transplants usually require an irradiation step. Irradiating the animals is the only way to replace the blood system of a mouse with donor-derived cells in an efficient, reproducible manner that can be compared with previous experiments and the large scientific literature. There is no alternative that can generate reproducible results, and efforts have been set to reduce to the minimum any suffering that may cause to the animals. By using a split-dose protocol, providing dietary supplements and enrichment to the cages, mouse welfare is significantly improved and re-established after irradiation.

On occasions, we will use treadmill exercise an alternative established and non-invasive model to stimulate the activity of the nervous system (avoiding the requirement of drugs).

In the rare case of invasive procedures with a larger impact, such as terminal bleeds and a subset of surgeries, the animals are anaesthetised prior to the procedure but not recovered after that, so that they will not experience any pain.

Also, all our studies imply the administration of substances (either cell populations in suspension or drugs). When administering a drug, we rely on our previous experience and the literature to know its expected undesired effects and determine the most appropriate amount and way to provide it. When sufficient information is not available for a specific study plan, a pilot study with a reduced number of animals will be designed to assess for potential toxicity and efficacy, starting with a low amount through the route with lesser undesired effects. In pilot studies, or if a drug is being administered to a particular mouse strain for which side effects may be enhanced, increased health checks will take place for closer follow-up. These measurements will allow to detect an unnecessary harm and to improve endpoints and reduce possible suffering.

Rarely, we might need to perform a surgery for an experiment. This might be to either install a minipump or pellet under the skin, if a substance needs to be provided for a longer period of time and this approach is considered to be the most appropriate for the animal’s welfare. Surgery is also needed

to implant an imaging window in the mouse skull to visualise blood stem cells in the animal, the only feasible way to study the interaction of blood stem cells with their home in real time. These experiments are only performed in a highly selected number of mice by highly skilled and experienced researchers who have perfected the technique for the animals to ensure a rapid and unremarkable recovery. Also, all our procedures are done in the cleanest possible conditions and in accordance with the Home Office Minimum Standards for Aseptic Surgery and the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgeries, to ensure we cause the least distress and lasting harm to these animals.

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

Mouse models have been used extensively in blood research because blood production is best understood in the mouse. Given the time needed for blood regeneration after transplantation or for blood cancer development, it is not possible to use immature life stages or terminally anaesthetise a mouse for the duration of the study.

Additionally, our group investigates the complex interactions between mammalian blood stem cells and their home, comprised by a multitude of different cell types but also structures (bones, vessels, nerves). It is currently not possible to model these complex interactions in less sentient (and less complex) species.

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

Experience has allowed us to refine our animal models and methods. Our current protocols are optimised based on our results and the feedback from the animal facility senior technicians, which allows us to reduce the impact on animal welfare while obtaining the necessary data from each mouse. Mice are housed with cage mates, in an environment with added enrichments such as bedding materials, tubes or playing items, to reduce their stress and ensure their socialisation and entertainment.

Furthermore, all mice undergo regular health checks adequate for each protocol and step. Early detection of any health concern allows us to make appropriate plans for those animals to prevent a worsening that could compromise their welfare, as well as to increase their monitoring. Health concerns are registered in an online database, which allows us to detect any possible unexpected side effect (or a major frequency or intensity for a specific mouse strain) and adapt the other studies accordingly to minimise the harms for the animals.

Mice with a genetically-modified immune system may be used as recipients for human cells. In order to study blood cancers in their full complexity and develop effective therapies, it is necessary to establish humanised animal models. This requires the use of mice with a defective immune system, which are capable of sustaining human blood cell production after human blood stem cell transplantation.

To generate transgenic mice, methods where any symptoms can be experimentally controlled, will be used when possible. The mice will be allowed to acclimatise to the animal facilities and should not display any symptom until we trigger the disease initiation. Most of the strains we use are not expected to present any adverse effects and the vast majority of our procedures performed are going to be

minimally invasive and are not expected to produce long-lasting harm. When unexpected symptoms arise (such as some deformities of the skeleton), the animals will be monitored and scored using sheets.

We find that strains that have been crossed with Nestin-gfp mice have an increased incidence (<5%) of developing skeletal deformity. The skeletal deformity appears as a “hunch” in the spine, sometimes accompanied by a waddled walk, most noticeable in the hind limbs. Most often, skeletal deformity is observed in older animals and more rarely in young animals. We are trying to breed this phenotype out of our colony by periodically refreshing breeders with wild-type mice. The animals with skeletal deformity will not be used as breeders but, if the deformity is mild, they might be used for downstream experimental protocols, to reduce the number of mice generated. Animals developing skeletal deformity will be recorded and monitored. We will health check the animals on a weekly basis and record their follow up in an observation sheet, to see if their overall health is affected (weight loss, impaired gait or reduced mobility). If the animal starts to lose weight or worsens the health checks will become more frequent, up to daily ones if needed. Mice will be killed by a schedule 1 method if they lose 10% of peak body weight.

In our experiments with animal models of blood cancer, we try to minimise the time that the animal is sick and during that time, we try to reduce the burden of the disease using therapies, in line with our research questions.

In the case of irradiation, we try to alleviate collateral damage and prolonged suffering by splitting the irradiation dose and to reduce the risk of infections and improve recovery rates by providing acidified water and prophylactic antibiotics (as advised by the Named Veterinary Surgeon, or NVS). Depending on the compound, pellets might be used for chronic studies as an alternative to drinking water, which can negatively affect the animal’s weight. In those cases, the animal's weight will be carefully and frequently monitored and recorded.

On occasions, we will use treadmill exercise as an alternative established and non-invasive model to stimulate the activity of the sympathetic nervous (avoiding the requirement of drugs). Only moderate exercise will be applied, never reaching exhaustion.

To reduce procedural harm, we will ensure that researchers will receive extensive training and only perform procedures when they have achieved competency, which will be carefully monitored and logged in databases. When appropriate, researchers in training will practise on dead animals and inanimate objects before handling live animals after having watched videos demonstrating the technique and experienced personnel. All researchers will be supervised until they are assessed as competent.

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

We will follow the PREPARE guidelines for planning animal experiments and improving reporting. These complement the ARRIVE guidelines containing the best practice and important information to include in publications describing animal research.

We will adhere to the Guidelines for the Welfare and Use of Animals in Cancer Research as set out by Workman et al. 2010 and implement the principles in the NC3Rs guidelines (including justification of species, details of power calculations and plans to minimise experimental bias). We will also follow the UKCCCR Guidelines for the Welfare of Animals in Experimental Neoplasia, including leukamia.

We will consider guidance from the Laboratory Animal Science Association, (LASA) https://www.lasa.co.uk/current_publications/ and will follow the Animal testing and research: guidance for the regulated community published by the Home Office on 26th March on 2013 and updated on 20th Oct 2022.

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

Both Project and Personal License holders will keep up to date about advances in the 3Rs by frequently consulting broadly accessible resources such as the Home Office guidance (https://www.gov.uk/guidance/research-and-testing-using-animals), the Laboratory Animals Science Association website (https://www.lasa.co.uk), NC3R's (http://www.nc3rs.org.uk), RSPCA (http://www.rspca.org.uk/sciencegroup/researchanimals) and UAR (http://www.understandinganimalresearch.org.uk/). Locally, we have access to updated information on Policies, AWERB, 3Rs Search Tool and training.

We organise weekly lab meetings and journal clubs to ensure that we keep up to date within relevant experimental animal work in our research field. Not just by following the latest publications, but also by taking part in reviewing articles and joining international and national conferences, we can keep track of the newest approaches other groups might be doing regarding animal research and ensure our methods stay updated within the new refinements implemented by others.

I will continue to participate in 3Rs workshops intended to guide other researchers on the use of substances that we frequently administer, and the optimisation of these protocols to minimise animal suffering.