Home Office

NON-TECHNICAL SUMMARY

Investigation of normal haematopoietic stem cell subversion and the evolution, maintenance and targeting of haematological malignancies

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

Normal blood stem cells, Blood cancers, Evolution and maintenance, Therapy, Resistance

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?

We will determine what goes wrong with normal blood stem cells in processes such as cell growth and survival and how abnormalities of these lead to the development and maintenance of blood cancers. We will then use this information 1) to help predict those likely to develop blood cancers, 2) to potentially intervene earlier in the course of blood cancer development and ideally prevent blood cancers from developing in those at high-risk and 3) to better treat those patients with established blood cancers.

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 cancers are devastating diseases that are associated with significant illness, with many being fatal. They represent around 1 in 10 of all cancers and may be preceded by early/pre-cancerous conditions. Blood cancers are relatively common when considered as a group, occurring at a frequency of approximately 95 cases per 100,000 of the population (Surveillance, Epidemiology and End Result (SEER) programme data) when the pre-cancerous conditions also included. Established blood cancers are often incurable and caused 12,961 deaths annually in the UK in 2015-2017 (CRUK statistics). Although they do affect the young, being the most common form of childhood cancer, blood cancers are predominantly diseases of the elderly and their incidence rises steadily with age. The age of the general population is rising steadily, and the prediction is blood cancers will become significantly more common over the next few decades. Moreover, some disease such as Non-Hodgkin's Lymphoma (NHL), the single most common blood cancer and the 6th most common cancer of all types, has been seen to be occurring more frequently than would simply be expected from the ageing of the population. Blood cancers are also not only associated with significant illness and death; although poorly effective in curing blood cancers, the treatments that we currently use are also highly toxic and extremely expensive (costing up to around £250,000 per patient if a bone marrow transplant, is used as part of the treatment). Thus, the treatment of blood cancers is an unmet medical need and costs the NHS more than £100 million annually.

This project is important as it aims to improve on the range and effectiveness of therapies for the treatment of blood cancers. It will do so by identifying new treatments for blood cancers, determining how resistance to existing and novel therapies develop and can be prevented and, through the study of early stages of blood cancers, will test the effect of and suggest the likely benefit of earlier therapy. Multiple solid organ cancers are more easily treated and sometimes cured by treatment earlier in the disease course and we hope that this general observation will extend to blood cancers. It is also possible that we may be able to, in effect, prevent certain blood cancers from occurring. Work over the last decade has demonstrated that the mutations that have been shown to cooperate to generate clinically obvious blood cancers can sometimes be detected earlier in apparently “normal” individuals with no apparent blood disorder. This condition is called age-related clonal haematopoiesis (ARCH) or clonal haematopoiesis of indeterminate potential (CHIP), and will hereafter be simply referred to as

clonal haematopoiesis (CH). Thankfully not all individuals who carry these mutations go on to develop a blood cancer, but the likelihood of this occurring is increased significantly in comparison to the general public and to those who do not carry these mutations. Although our understanding of how individual mutations contribute to the maintenance of established blood cancers is improving, with our own groups contributing significantly to that understanding, exactly how they change the function of the normal blood stem and progenitor cells that generate all the different blood cell types is poorly known. Similarly, the progressive events that lead to the development of newly diagnosed and treatment-resistant blood cancers are very poorly understood, and this lack of knowledge hampers our attempts to improve treatment outcomes.

Blood cancers originate in haematopoietic (blood) stem cells (HSCs). These cells have the ability to remake themselves by dividing to generate daughter cells with exactly the same properties; a process known as “self-renewal”. This ability may be an in-built feature of the cell (such as in a normal HSC) or it may be acquired, i.e. due to the effects of a mutation occurring in that cell. Like all cells within the body these stem cells are constantly exposed to external stresses and influences that can lead to the development of mutations. These mutations alter the code of DNA and through this the information contained within DNA to control the behaviour of the cell. Thankfully, the vast majority of these mutations are fully repaired, however small numbers of mutated cells can persist. Unlike the majority of other cell types that simply mature and eventually die, HSC possess self-renewal and can therefore retain the mutations within their DNA by passing them onto their daughter cells. Whilst the vast majority of such mutations are “neutral” and do not affect the behaviour of the HSC, certain mutations can equip an HSC with an advantage over time, allowing its daughter cells to expand, which gives rise to clonal haematopoiesis (CH). CH is driven by a group of mutations that are associated with the development of blood cancers, although this progression is not inevitable. Importantly, CH becomes increasingly common with age and is detected in more than 30% of people aged 70 years and older. Similarly, specific long-lived stem-like progenitor cells within the immune system that carry immunological memory are also thought to develop mutations and predispose individuals to the development of the blood cancers derived from these tissues, lymphoma and myeloma.

In addition to mutations changing the internal behaviour of HSCs, it is clear that other factors externally influence the behaviour of HSCs during normal blood cell development and during the development of blood cancers. There is now increasing evidence that the rate at which an HSC and its daughter cells grow, is affected by ageing and external “stress” factors such as infection, inflammation and diet. HSC and progenitors reside in a specialised environment within the cavities of bones. This environment is called the bone marrow and the non-blood forming cells that HSC interact with are known as the bone marrow microenvironment (BMME). The BMME provides a protective and nurturing environment for the continued growth of HSC. It is also thought likely to produce the final signals of the above “stress” factors that directly stimulate the HSC and, in part, facilitate the development of blood cancers. Moreover, it is thought that the BMME also provides survival signals to blood cancers once they develop and is a critical factor in the development of treatment resistance in blood cancers. However, how these signals change from the support of normal HSC to the support of blood cancer stem cells is unknown.

This project will investigate internal (intrinsic) and external (extrinsic) factors that allow the normal function and growth of HSC and how these factors are altered to initiate and maintain blood cancers, with the aim of identifying therapeutic targets to intervene to improve outcomes or potentially even prevent the formation of blood cancers.

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

The new outputs that we expect to see from this project will relate to new knowledge obtained about normal blood stem cell function and how this is altered to generate blood cancers. This information will also include identifying specific critical factors that control this process that might be useful as therapeutic targets, to treat or potentially prevent the development of these cancers, or as markers to diagnose them earlier in their development. As our project will involve therapeutic targeting of these candidates, we also aim to identify potential novel treatments that act on the targets and to generate enough data on their effectiveness to enable the treatments to be taken forward into clinical trials.

Our project will also generate new strains of genetically altered animals to be used as models for studying blood cancers which will be of great potential advantage to other researchers studying the same disorders.

We will publish our results and data with free access in reputable scientific journals and talk about it at major conferences. We will also deposit our data in standard archives so it is available to other researchers and can help to guide their studies. Our research will also be of wide public interest and important to a number of major charities and patient groups and we will communicate our findings as broadly as we can, through press releases and by contributing to events organised by those groups.

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

In the short-term, the medical and scientific communities, as well as drug manufacturers, will benefit from our research by using our findings to help develop new strategies to identify, potentially prevent and treat blood cancers.

To facilitate the rapid benefit of patients, we plan to work along with other scientists, medical doctors and biomedical companies to speed up the application of our findings into new testing methods and new treatments. We have a great deal of experience in such processes and we are already advanced with trials of drugs discovered through similar studies in previous licences.

In the longer term, we hope that our work will lead to the development of new methods for the prevention and treatment of blood cancers. Prolonged survival and a better life quality for patients with blood cancers and their families will be the most direct and measurable benefits from our research. In the broad sense, ensuring better treatment options for patients will have a positive effect on society at many levels.

Our groups have a longstanding interest in public engagement activities and we work in or are associated with collectives and in institutions that have dedicated public engagement teams. We will remain actively involved in these activities to promote the understanding of our research, in line with the programme of public engagement proposed by our institutions.

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

Our findings will be made available to other scientists through publication in peer-reviewed journals and presentations at scientific conferences and meetings. The generated data will be stored in an archive and made freely available to other researchers around the world via databases such as the

European Nucleotide Archive (ENA) at the European Molecular Biology Laboratory- European Bioinformatic Institute (EMBL-EBI). Our new animal models, as well as tissues collected from the mice will be valuable to other scientists studying the development of blood cancers. We will distribute our mice and other tools freely to non-commercial establishments after publication. Pre-publication access will be extended to other scientists as part of collaborative studies. We have already exported a number of our mouse models to several leading universities and institutes worldwide. We will also aim to share and use tissues from aged mice using ShARM (Sharing Ageing Research Models, https://www.sharmuk.org).

Species and numbers of animals expected to be used

- Mice: 36,900

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 widely used for biomedical research due to their anatomical, physiological and genetic similarity to humans. Furthermore, they are easy to breed and keep in a laboratory environment. The enormous progress of technology over recent years has enabled us to efficiently generate mouse models useful to study disease states such as the blood cancers that are our main research interest. Our mouse models carry changes in their genetic material (mutations) copying or resembling the changes in genetic material of patients with blood cancers.

The incidence of blood cancers increases with patient age. Consequently, the effects generated by the ageing on the blood system are an essential part of our project. In many aspects, the ageing of mice resembles the ageing of humans and therefore, mice are an extremely useful resource to understand human ageing. The life-span of a mouse averages around 2 years which allows us to complete the project goals within a reasonable time-frame. Equally, we will use young adult mice as a comparator and reference for results obtained with aged mice.

Our work is focused on the blood system and will benefit from a well-established specific range of methods that are already established and accepted for the mouse. For example, blood cells can be easily analysed and separated by flow cytometry, a method where various molecules on the surface of the cell are specifically tagged and caused to emit light under certain conditions. This labelling can be subsequently used to identify and separate out the cell for further analysis.

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

Most methods and procedures required for this project are well established in the field of blood cancer research and in our group. We have successfully used them in the past to characterise different mouse models of leukaemia and lymphoma.

In practice, mice will be bred to generate offspring that have alterations (mutations) of relevance in their genetic material (DNA), so-called genetic alterations. For the wellbeing of the animal, we normally engineer these alterations under our control, so that a mouse will need a specific “switch” involving a single or repeated injection of a substance that will turn the genetic alteration on or off. Some of these experimental mice, after activation of genetic alteration, may be kept alive until up to 2 years of age to assess the role of the ageing process, an independent risk factor in blood cancer formation. Throughout this time, we will collect blood from mice on one or multiple occasions to assess how the number of blood cells change, as one of the markers to assess disease progression in response to various treatments, either to potentially stimulate the development of blood cancers or as potential treatments for these cancers. Frequently, these treatments will aim to mimic the exposures normally encountered by humans during their lifetime, such as inflammation following infection or DNA damage. The treatments may last up to 16 weeks and are expected to produce long-lasting, but generally immediately undetectable changes, in the same way that occurs in humans. To this end, mice may be treated with different substances to modify either the behaviour of the abnormal blood cells or their normal body environment, the bone marrow microenvironment (the surrounding cells that support the growth of blood cells within the bone marrow). The treatment may involve a single or multiple administrations of a substance by different delivery routes (e.g. in the food, injected under the skin or into the body). The frequency of these treatments and volumes used are not expected to harm the animals. Examples of cell-targeting substances include drugs that have been shown to effectively inhibit tumour cell growth when tested "in a test-tube". Some mice will undergo treatment with radiation, either as a challenge to the haematopoietic system, and/or to remove their own blood system (myeloablation) before this is restored through transplantation of blood cells from another mouse. Transplantation will be performed only once in a life time of a mouse. Depending on the amount of radiation given, mice may experience post-irradiation illness but our experience with this situation will effectively limit this condition. Human blood cells may be transplanted into special mice that lack an immune system and tolerate the growth of these cells to confirm the findings from studies in mice or to identify subtle areas of difference between humans and mice.

Mice will be killed by one of the approved humane killing methods. Upon killing of a mouse, we will collect different organs (e.g. bone marrow, spleen, liver) to analyse multiple areas of interest, such as proportions of different cell types, and what genes and proteins are switched on in the cells.

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

General health monitoring is performed for all mice by trained personnel, both in the animal facility and in our own research team. Given our long-standing experience with these mouse models of blood cancers, we have an in-depth understanding of the possible adverse effects of our experiments and can enhance animal care for the mice at increased risk of ill-health. In addition, when visible signs of sickness or distress are observed, enhanced monitoring and support will be put in place according to the care plans included in this license.

During our project we will use different procedures and experimental setups which are expected to have various levels of impact on animal wellbeing as listed below.

1. Breeding of genetically altered mice is not expected to generally cause harmful effects. Nearly all of the genetic changes used for our project will be seen in the adult animals used for our experiments, by which time they will usually have been moved to an experimental protocol. Therefore, the majority of

the animals used for breeding will not experience adverse effects which might arise from changes in their blood system.

2. Induction treatments, such as injections of pIpC or tamoxifen which trigger switches within the mouse cells, are given to activate genetic alterations. Activating substances will be injected in mice up to 7 times, given every day or every other day. A mild weight loss up to 10% of pre-treatment weight may appear in relation to biological effects of injected substances. These adverse effects will decrease or stop once the induction period is finished and will not affect the mice long-term.

3. Blood collection will be used to obtain samples for tracking of normal blood formation and disease progression and other analyses. This will be performed up to 20 times during the life time of a mouse, with at least 2 weeks between the bleeds. The bleeding may lead to short-term discomfort and stress but this will be minimised by the preferable use of saphenous vein sampling and is not expected to affect the animal in the longer-term.

4. Blood cancer will develop in some of the mice. Over weeks or months, these mice usually show gradual changes in the number of blood cells, which may cause an increase in the size of the spleen and occasionally, in liver size. In studying lymphoid malignancies, swelling of the lymph glands or other tumours may also occur. Their growth will be judged by external signs such as swelling of the abdomen or the appearance of masses under the skin. Occasionally testicular swelling may be apparent. Mice may appear hunched and scruffy and their hair may stand on end and they may show reduced activity towards the advanced stages of the disease. These signs will be collated and will be included in the humane endpoints.

5. Transplantation, a transfer of blood stem cells, will be performed in some of the mice. This is a standard method to assess the potential of cells to regenerate the whole blood system in a recipient mouse and represents a massive challenge to blood cells. The tail vein will be used as the most frequently route via which to inject the transferred cells. A transient discomfort and stress due to a temporarily handling of the animal, as required for injection, may lead to a short-term discomfort and stress-induced behaviour. However, the procedure itself is not expected to negatively affect the animals in the long term.

The injection of abnormal blood cells into the cavity of one of the long bones (e.g. thigh bone) may be used in specific cases to improve the efficiency of the transplantation or to modify the experimental outcome. This is a surgical procedure and is discussed below, under a paragraph 7 about surgeries.

6. Preconditioning (preparation) may be needed for some mice before they have transplantation. Irradiation will be used most frequently, but other methods may be used as well. Irradiation may induce a post-irradiation illness and the level of the illness depends on the amount of irradiation (the dose) received by a mouse. The doses will always be adjusted to ensure the least possible dose is used, therefore limiting animal suffering.

- Low dose of irradiation may cause a mild drop in weight of mice (up to 10% of pre-irradiation weight). The reason for this is predominantly a decreased interest in food and water, leading to poor eating and dehydration. The activity of mice should remain unchanged. Irradiated animals will be checked daily and weighed at least twice weekly to monitor their health. An enhanced pre- and post- irradiation care plan will be provided if necessary. Our experience and the published data show that the mice recover

fully from the irradiation-related adverse effects, usually within 7-10 days. The mice should return to their weights within 7-10 days post-irradiation.

• High dose irradiation leads to a long-term failure of the entire blood system and therefore, the irradiation induced ill-health is more pronounced. Mice may drop in weights by up to 15% of their pre-irradiation weight. Moreover, mice may be less active. The mice should return to their weights and activity within 7-10 days post-irradiation.

Sometimes, only a part of a mouse body will be irradiated. These mice will be put under for 20-30 minutes, using an anaesthetic (inducing a state of a deep sleep). This short anaesthetic will not result in long-term adverse effects.

Preconditioning will be performed only once in the lifetime of a mouse.

7. Surgical procedures will be performed in aseptic conditions to prevent infection and under anaesthesia. Following surgery, mice will be kept warm until they are fully recovered and moving freely around the cage. The mice are expected to recover from anaesthesia within 20-30 minutes at most and no long-term effects are expected. Medication to relieve any pain will be administered to the mice while they are anaesthetised for the surgical procedures. Mice should be fully active within 2 hours from surgery. Surgeries should not result in long-term adverse effects. Difficulties to regain activity beyond 2 hours post-anaesthesia or signs of more than mild pain and distress will be humane endpoints.

8. Imaging may be performed on some mice to visualise blood cancer growth. Injection of a substance for imaging will be performed before each session. Each session will last up to 45 minutes. At most, each animal may undergo a maximum of 15 imaging sessions in total, with no more than three imaging sessions per week Imaging will be done under anaesthesia (a state of deep sleep) and the mice are expected to be fully active within 20-30 minutes post-anaesthesia. Imaging is not expected to result in long-term adverse effects. Animals will be allowed to fully recover from anaesthesia in between sessions and will only be imaged if they are deemed to be fit enough for the imaging session.

9. Different treatments will be used to alter positively or negatively the progression of the blood cancers under study. We will use only substances with known properties and known to cause up to moderate adverse effects such as reduced activity, decreased feeding and weight loss up to 15% of pre-treatment weight. These adverse effects may persist throughout the whole period of treatment. The mice may receive the substances in drinking water, by specific feeding (gavage), in food or by injections.

10. Physiological treatments that we expect will positively or negatively alter the progression of blood cancers will be used to mimic states normally met during a human lifetime. Examples of such treatments include a nutrient-deficient (folate) diet and inflammation. These treatments may result in mild disturbances to the normal condition of an animal, such as mild changes in their activity and weight gain or loss up to 10% of their pre-treatment weight. These treatments will last up to 12 weeks.

11. Ageing is a physiological process and is not expected to have more than mild effects on mice. General physiological features of ageing such as reduced activity, weight change, hair loss or occasionally blindness may appear and persist until the animal is killed. The ageing mice will benefit from an enhanced care plan and be killed immediately if they experience any signs of suffering. Rarely, different cancers may develop in the oldest animals and an existing blood disease may progress faster in aged mice.

Unless otherwise specified, the work in this licence will be undertaken in accordance with the principles set out in the Guidelines for the welfare and use of animals in cancer research. British Journal of Cancer (2010) 102, 1555-77.

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)?

Below is the summary of animal numbers used on different severity protocols:

Moderate severity, 10950 mice:

Protocol 2: Embryo recipients 400 - all mice will reach moderate severity

Protocol 3: Vasectomy 100 - all mice will reach moderate severity

Protocol 6: 4000 moderate - around 10% predicted to reach moderate severity (400)

Protocol 7: 5000 - 25% predicted to reach moderate severity (1250)

Protocol 8: 3000 - all will reach moderate severity (3000)

Protocol 9: 4000 - all predicted to reach moderate severity (4000)

Protocol 10: 4000 - 50% predicted to reach moderate severity (2000)

Mild severity, 15,400 mice:

Protocol 1: Superovulation 400 - all mice will reach mild severity

Protocol 5: Breeding 15000 (this number includes animals that will be moved to other protocols)

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

- Killed
- Used in other projects

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 formation of blood cells is highly complex. It is not possible to fully replicate its baseline function or how this is altered during the evolution of blood cancers or how these developing cancers respond to treatment using test tube experiments in laboratories. Only by studying living models can we reach reliable conclusions and produce evidence that will permit our research to advance to studies in man.

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

To answer our scientific objectives, we always utilise available data within the public domain, make extensive use of cell lines and perform non-regulated laboratory studies in the test tube on human and animal tissue that will minimise work in living animal models. With a collaborator, we have recently established mesenspheres, three-dimensional systems that include co-cultures of both blood cells (abnormal or normal) and cells derived from the bone marrow microenviroment. These better mimic the situation in animals by providing some of the external signals that we wish to study. Taken together, these strategies have already allowed us to replace some animals required for this project.

Why were they not suitable?

These experimental systems do provide useful data that supports and is complementary to experiments in living animals. However, they lack the complexity to accurately reproduce and represent the whole blood forming system of a living animal.

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 used the experience of our previous projects to estimate the numbers of animals required. These are also based on the predicted growth in size of our respective groups and the shift to perform more translational/therapeutic experiments. This project has a moderately increased scope on our previous licence, including increased complementary work on lymphoid malignancies and a greater focus on the earlier stages of malignancy development where the changes are more subtle and larger numbers of mice may be necessary. Therefore we anticipate a slightly greater number of experiments. However, the measures we will take, as described below will allow us to reduce the average number of animals required per experiment. Our estimated numbers are thus substantially in line with our previous Project Licence.

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

We have employed several advances in practice, including the NC3Rs experimental design assistant, in designing our experiments which have allowed us to reduce the number of animals being used in

this project.

- Tissue collection: Previous general practice has been to harvest bone marrow from the long bones of the lower limbs of mice. This typically provides around 10-40 million cells per mouse. We will be employing a newer methodology that uses almost the whole skeleton to provide bone marrow and can yield 100-400 million cells per mouse. This will allow us to reduce the number of mice that we need to use for such tissue-based experiments by approximately 50%.

- Bioluminescence: The main method previously used to assess tumour growth kinetics was white blood cell count. Adopting bioluminescence as a method of assessing tumour growth will allow this to be performed with greater accuracy, reliability and efficiency. Since development of disease can be investigated longitudinally, the use of imaging methods will also allow a reduction of numbers of mice required. We will genetically alter tumours to express the luciferase gene prior to transplantation. Administration of a substance, luciferin, is used by luciferase to emit light from the luciferase containing cells, that can be detected by sensitive cameras. The light emitted is therefore directly proportional to the number of cells that contain luciferase and to the tumour size. The greater accuracy and reliability with which we can assess therapeutics and other alterations that validate potential therapeutic targets has also allowed us to reduce the number of mice required.

- Increased use of injections directly into the long-bones of the animal to generate bone marrow transplant recipient. This will result in a more efficient and reproducible process and will therefore require fewer donor animals.

- Experiments have been designed based on previously published studies as well as current manuscripts, grants and ARRIVE guidelines. Published studies and grant awards have been thoroughly peer reviewed, including by statisticians, to help ensure numbers of animals are reduced as far as possible

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

We will take all possible measures to optimise the number of animals on our project. Examples include:

- Sharing tissue within our institution.

- Collaborating with other researchers and consulting published data to avoid strain and experimental duplication.

- Employing pilot studies with small numbers of animals to optimise experiments and therefore reduce the total numbers of animals required to answer a specific research question.

- Employing the most efficient breeding techniques, including timing, rotation and good husbandry.

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.

Our project uses mice as a model of the blood forming system and of blood cancer formation. The mouse is the most appropriate and most widely used model in this field. The methodologies used are very well established and have been refined to cause the minimum pain, suffering, distress and harm to the animals. An example of this is saphenous vein sampling rather than tail vein sampling to provide blood samples. The saphenous vein is larger than the vessels in the tail and can actually be readily punctured for sampling, as in a human blood test from a large vein in the arm, rather than tail vein bleeding where the blood seeps out as it would from an actual wound. The majority of the protocols have mild impact on the animals. Protocols which have a greater impact on the animals are only used when there is no alternative. We will also make use of analgesia on all procedures where this is readily available and appropriate (e.g. intrafemoral injections).

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

Adult mice are used in our project as the least sentient species from which meaningful experimental data can be generated. They share sufficient physiological and pathological traits with humans and yet are less sentient than other mammalian models. Because mice are the most appropriate and most widely used model, our data can be compared with other researchers', increasing the value of our studies to the scientific community as a whole. As blood cancers often require extended periods of time to manifest, it is not applicable or appropriate to use terminally anaesthetised animals.

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

We will follow best practice as established locally and follow national guidance by e.g. NC3Rs to minimise the welfare costs to our animals. Examples of best practice we will employ include:

- Anaesthetic and pain medication will be applied when animals undergo surgical procedures.
- Needles will only be used once. This reduces infections sustained by animals and ensures that only sharp needles, which cause less pain, are used.
- Oral gavage, a procedure whereby blunt-ended needles are used to introduce controlled volumes of liquids into the stomachs of mice, mimicking oral administration of drugs, will be used to administer potential therapeutic agents to remove the need for injections.

- A bioluminescence methodology whereby tumours are genetically altered to express the luciferase gene prior to transplantation. This allows the accurate assessment of tumour growth kinetics by non-invasive imaging in living animals, removing the requirement for blood sampling and the greater handling required for clinical assessment.
- Nail clipping will be employed on mice exhibiting skin irritation to prevent overgrooming and scratch wounds.
- Facilities with special restrictions preventing the introduction of infection will be used to protect the health of immunosuppressed mice.
- Appropriate dosages will be calculated following extensive experimentation on cell lines.
- The health of all animals will be monitored at least daily, with particularly close monitoring of immunosuppressed mice for the development of opportunist infection.
- Whenever possible (e.g. in haematological malignancies that present with distinct and consistent peripheral blood abnormalities), serial blood counts will be used to predict endpoints.

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

We will follow established best practice guidance in planning and carrying out our experiments, re-visiting the guidance as it is updated. In particular we will follow the PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines produced by NORECOPA and the Guidelines for the Welfare and Use of Animals in Cancer Research produced by the National Cancer Research Institute (Workman et al, British Journal of Cancer, 2010) and the LASA guiding principles https://www.lasa.co.uk/wp-content/uploads/2018/05/Aseptic-Surgery.pdf). We will also follow the ARRIVE guidelines when planning and reporting our animal studies (https://arriveguidelines.org/arrive-guidelines).

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

Animals at our establishment are cared for by a team of highly-trained professional animal technicians who regularly review and update their practice to include advances in the 3Rs. Advances in 3Rs are communicated through user group meetings and written communication. Furthermore, we will seek out information on advances in practice from national sources such as the National Centre for the Replacement, Refinement and Reduction of Animals in Research (https://nc3rs.org.uk/the-3rs). In particular, we will take advantage of their webinars, e-learning and other online resources. We will also make use of international resources, such as the Humane Endpoints website of the 3Rs Centre at Utrecht University. (https://www.uu.nl/en/organisation/3rs-centre) and Norecopa, Norway's 3R centre and National Consensus Platform for the Replacement, Reduction and Refinement of animal experiments (https://norecopa.no).