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NON-TECHNICAL SUMMARY

Characterisation of normal Haematopoietic Stem and Progenitor cell function to understand and target mechanisms of haematological malignancy initiation, maintenance and relapse

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

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult, Aged animal

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

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 the licence is to identify how normal blood stem and progenitor function is corrupted, leading to the development and maintenance of blood cancers, and their relapse following therapy and how these longitudinal processes might be therapeutically targeted.

A retrospective assessment of these aims will be due by 23 September 2031

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve its aims and if not, why not?

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?

The Blood system is one of the most important systems in our bodies and is critically required for our continued survival. Our blood system performs a number of critical functions that keep us alive; the circulation, with blood as its carrier provides nutrients, soluble proteins and necessary cells to other parts of the body, it carries oxygen to tissues for respiration, energy production and biochemical purposes and also generates the cells of the innate and adaptive immune systems that help us fight infections and fight cancer. The blood system also makes the cell components, platelets, that form blood clots and prevent us from life-threatening bleeding. When Blood formation does not function properly, it generates serious illnesses; both malignant (cancer) and non-malignant (not relating to cancer). These can include life-threatening blood cancers like the acute leukaemias and malignant lymphomas that will be studied in this work. Blood cancers account for about 10% of all cancer diagnoses and are more common with age and collectively affect approximately 57 people per 100,000 of the population. As the world’s population ages, this number will increase, making it a larger health

and societal problem. Unfortunately, current treatments still aren't very effective and are often very toxic, and most patients have a poor quality of life and often die from these diseases or have significant side-effects if they do survive. A lack of understanding of how these cancers start and progress has hampered our recent progress. We propose to address this knowledge gap, by uncovering the causes of these cancers, which will, in turn, allow us to discover better, safer and kinder treatments. We also want to prevent these cancers from happening in the first place. In the past, prevention wasn't possible because people often got sick suddenly, without warning signs. However, recent research, including our own, has shown that it's now possible to identify people at high risk of developing these cancers years before they become unwell. This gives us a chance to find ways to stop the disease before it starts.

These cancers begin in blood stem cells, the single cell type that generates all of the mature blood cell types that carry oxygen, fight infection and cause the blood to clot. Blood stem cells are responsible for producing all the blood and immune cells we need throughout our lives. Over time, these stem cells can become damaged and as part of this damage can develop mutations (alterations in their genetic material). Most of the time, these mutations don't change how the cells work and are often lost through cell death. However, sometimes, the mutations provide the stem cells with an advantage, allowing them to grow faster and outnumber normal cells. This condition is called clonal haematopoiesis (CH). CH becomes more common as people age, affecting over 30% of people aged 70 and older. While most people with CH won't develop cancer, they do have a higher risk. We are interested in how these mutations provide an advantage and believe that this knowledge can inform the diagnosis of blood cancer earlier, can provide early interventions to prevent the progression of leukaemias and can inform the treatment of leukaemias and lymphomas at diagnosis.

Apart from mutations, other factors can affect how blood stem cells behave. Normal ageing/exposure processes or lifestyle "challenges" such as ageing, infections, inflammation, diet, alcohol, smoking, exercise, sleep and even the microorganisms that colonise our bodies all potentially play a role, and for some mutations, age has a particularly strong effect. These mutations may seem harmless in younger people, but they can lead to cancer in older people. However, we don't yet understand how these factors interact with the mutations to cause cancer. One of the aims of this project is to study these interactions. By learning more about what triggers the growth of mutant stem cells, we hope to find ways to stop the process and prevent cancer.

Patients with Blood Cancers are often initially sensitive to the treatments that we give them however, in the majority, the cancer comes back. This "relapse" is usually accompanied by the cancer cells being resistant to the therapy that was previously given and often to other therapeutic options also. Unfortunately, our understanding of how resistance forms and how to target this is very poor. Our project will specifically identify such mechanisms of resistance and will find ways to target these mechanisms to treat patients better after they relapse and, more importantly, to improve initial treatments to prevent resistance development and relapse in the first place.

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

This project aims to understand what goes wrong to allow blood cancers to occur and progress. This knowledge will allow ourselves and other researchers to identify processes that might be targeted with drugs and/or lifestyle changes to:

1) Prevent blood cancers from developing or progressing to more aggressive forms.

2) To more effectively treat blood cancers if they do develop

3) To effectively treat blood cancers if they re-occur, which is common and usually fatal

Moreover, knowledge from the project will identify those patients along this pathway of blood cancer development who will most benefit from treatments and/or preventative strategies. This will be facilitated through dissemination of our findings to the scientific community (e.g. attending and presenting on national and international conferences, press releases, publication of manuscripts on scientific journals etc.) and bench-to-beside activities (e.g. patent applications, clinical trials and collaboration with pharmaceuticals etc.).

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

Our work has a direct clinical benefit. The people most likely to benefit early from our findings will be doctors, scientists, and pharmaceutical companies, where we will help them with new ways to detect, prevent and treat blood cancers.

The group that we most want to help, and quickly, are patients. To speed up this process, we will further work with other scientists, medical doctors and biomedical companies outside of this project to turn our findings to identify new treatments and to improve current ones into standard-of-care therapies. We have a track record in this area with some of our previous findings from similar previous projects having identified new drugs and new combinations to treat patients with blood cancers.

Our longer-term aims will be to identify ways to prevent blood cancers from developing and/or to slow their progression, to also improve patients' outcomes. We also aim to understand the process whereby blood cancers become resistant to therapies and recur. Achieving these aims will mean that patients can live longer and have a better quality of life, which will also help their families and lessen the impact of these blood cancers on the NHS. Overall, preventing and improving treatment for these cancers will be good for our society in many ways.

The final group to benefit from the outputs of our work will be the general public. We are passionate about sharing our work and its implications with the general public and feel that their buy-in will greatly improve and broaden its impact. Our group ethos also perfectly aligns with the public engagement goals of our institution.

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

To maximise the outputs of our project we will share our findings with a number of stakeholders; with other scientists and doctors by publishing our results in high-impact scientific journals and by presenting them at scientific conferences and meetings, locally, nationally and internationally; with patients, their relatives and the general public through presentations, blogs and social media directed at these groups; with the pharmaceutical industry via a number of existing contacts; and with policy makers and health care providers, such as the NHS, as appropriate. The datasets that we generate from this project will be stored securely within our group and institution and following publication will be able to be accessed by other researchers around the world via data repositories such as GEO, the

European Nucleotide Archive (ENA) at EMBL-EBI and PRIDE, dependent on the type of data. Finally and importantly, we will make use of public repositories such as bioRxiv to communicate our results before peer review in scientific journals or to communicate ‘negative results’ (e.g. when therapeutic approaches were less successful than anticipated).

Any new animal models that we create, and the cells collected from these mice are likely to be helpful for other scientists studying blood cancers or potentially other cancers. We will freely share our mice and other research tools with non-commercial organisations after our findings are published, through material transfer agreements conducted via our University. For collaborative studies, we will also allow other scientists to use our models before publication.

Species and numbers of animals expected to be used

- Mice: 24000

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 close biological similarity to humans. This is particularly evident within the blood forming system and mouse models have been used for decades to study the formation, evolution and treatment of various blood cancers. Furthermore, they are easy to breed and keep in a laboratory environment. Advances in technology have also allowed us to easily and accurately stably modify the DNA of mice 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 and thus serve as useful models to study how blood cancers occur and progress and for the development of therapies to treat blood cancers.

Our work will require neonates, juveniles and pregnant adults, as well as adults and aged adults which are essential in order to achieve our scientific goals. The incidence of blood cancers increases with patient age and its role in blood cancer development will also be analysed within the project. In many aspects, the ageing of mice also closely 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, as opposed to that of a human (70-80 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 focussed on the blood system and will benefit from a well-established specific range of methods that are already established and accepted for use within the mouse. For example, blood cells can be easily analysed and separated by flow cytometry, a method where various proteins on the surface of the cell are specifically tagged. 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 of the methods and procedures needed for this project are well-established in blood cancer research and within our group. We have successfully used them in the past to study different mouse models of pre-malignant states, leukaemia and lymphoma.

In practice, we will breed mice to produce offspring with specific 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. To activate these genetic changes, we sometimes use a procedure that involves one or more intraperitoneal ((i. p.), into the peritoneal cavity) injections of a substance to turn the genetic alteration on or off. In some of these experiments, mice may live up to 125 weeks after genetic activation, to assess the role of the ageing process, an independent risk factor in blood cancer formation. During this time, we will collect blood from the mice from tail vein or saphenous veins (located in the legs) at various intervals to monitor how the number and types of blood cells change, as a marker of disease progression in mice carrying one or several genetic alterations. If a large volume of blood is to be analysed, we will harvest this via terminal cardiac puncture. This is a bleeding method whereby blood is collected directly from the heart, a terminal procedure that is conducted under deep anaesthesia, and the animal will then be humanely killed. Similarly, terminal anaesthesia may be used to perfuse the animal with a fixative solution to preserve its organs for further analysis.

To investigate how factors that mimic the real-life human exposures implicated in the initiation and progression of blood cancers, such as infections, inflammation, diet, ageing-related changes, toxins, and other external or internal influences, alter the incidence of leukaemias and lymphomas, we will treat some experimental mice with substances that mimic acute infection (for example, one dose of intraperitoneal injection of poly(I:C), chronic infection ((i.e., multiple doses of intraperitoneal injection of poly(I:C) or bacterial cell elements such as LPS)), low-grade inflammation (high-fat diet or other unhealthy diets) or substances that cause abnormal activation or inactivation of proteins that are associated with blood cancers, or substances that mimic ageing related factors. These treatments may last up to 12 weeks and are expected to cause long-lasting changes in stem and progenitor cell properties. However, the extent of these changes may be subtle, and similar to those observed in humans exposed to these factors repeatedly during their lifetime. We will also study the effect of anti-inflammatory treatments with various substances to modify the behaviour of the altered blood cells or their environment. The treatment may involve single or multiple administrations of a substance through different delivery routes (e.g. in food, drinking water, via oral gavage or subcutaneous or intraperitoneal injection). The frequency and volume of these treatments are not expected to cause long-term harm to the animals. Additionally, to mimic other life-style differences, mice may undergo behavioural modifications, such as changes to their light-dark cycle, a modified diet, or exercise equipment such as treadmills, rotarods and grip meters, to indirectly influence blood cell activity.

Transplantation will be performed only once in a mouse's lifetime. Transplantation involves injecting donor cells into recipient mice either via a non-invasive route, such as tail vein injection, or into the marrow cavity of a bone like the femur under anaesthesia). Before receiving a donor cell transplant from another mouse, some recipient mice will undergo irradiation or treatment with a chemotherapeutic agent to weaken or remove their own blood system, so the host bone marrow cells do not compete with the donor cells for space, allowing donor cells to efficiently engraft (settle and regrow the blood system within the host bone marrow) and produce blood and immune cells. Depending on the amount

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of irradiation, mice may experience post-irradiation illness, but we are very experienced in this procedure and our advanced care plan will effectively manage this condition. When relevant, human blood cells may be transplanted into mice to confirm findings from mouse studies and/or test therapies on human cells. The transplanted cells may also be pre-manipulated in vitro (outside the body, usually in a petri-dish used in laboratories) to enhance or hinder their potential to generate leukaemia in mice. Occasionally, cells may also be further manipulated to emit light (bioluminescence), enabling imaging of cell growth within the body and to repeatedly measure tumour growth accurately without killing the mice. Upon injection of an imaging substance into the mice, this light can be captured from living mice placed in an imaging system. To ensure stress-free restraint during imaging, mice will undergo short-term anaesthesia and are expected to recover without long-term harm.

Anti-leukaemic treatments may be administered to prevent or slow down growth, or to eliminate leukaemic cells altogether. These treatments may involve repetitive handling of mice for brief moments to administer the substance by oral gavage or by injection. The frequency and volume of these treatments are not expected to harm the animals long-term.

As blood cancer-linked mutations are sometimes found to develop before birth, to understand how they affect the blood system in early life, sometimes we may inject manipulated cells (such as embryonic stem cells edited in a petri dish) into early-stage embryos to study how the manipulated cells affect early-stage blood system formation.

At the end of experiments, mice will be humanely killed. After euthanasia, we will collect many different organs (e.g. bone marrow, spleen, liver, lymph nodes, thymus) to analyse multiple parameters such as the proportions of different cell types, gene expression and protein expression.

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

Highly trained personnel in the animal facility and our research team will perform regular health monitoring for all mice to ensure their optimal welfare. With our extensive experience in studying mouse models of blood cancers, we understand the potential adverse effects of our experimental designs and can enhance care for mice at increased risk of ill-health. Any mice showing signs of ill-health or significantly reduced activity will be humanely killed within 24 hours depending on severity and the type of the clinical signs. In addition, when visible signs of compromised well-being (such as piloerection characterised by the body fur standing on end, prolonged hunching, inappetence, reduced activity for more than 2 hours) are observed, enhanced monitoring and support will be implemented, according to the detailed care plans included in this license.

During our project we will use different procedures and experimental setups that are expected to have varying levels of impact on animal well-being as listed below, with the major expected impacts and/or adverse effects given.

1. Breeding of Genetically Altered Mice:

Breeding genetically altered mice is not expected to cause harmful effects. Most genetic changes used in our project will manifest in adult animals used for experiments, after the “switching on” of genetic manipulations, therefore the majority of breeding animals will not experience adverse effects from changes in their blood system.

2. Induction Treatments:

To activate genetic alterations, substances will be injected into mice up to seven times, either daily or every other day. Mild weight loss of up to 15% may occur due to the biological effects of the injected substances and/or routes of administration. When tamoxifen is administered via injection under the skin, abdominal swelling and scrotal swelling may be seen. These adverse effects will subside once the induction period ends and will not have long-term effects on the mice.

3. Blood and Bone Marrow collection:

Blood samples will be collected from a superficial vessel such as the tail or saphenous vein up to 20 times during a mouse's lifetime. Sample volume will not exceed 10% of total blood volume in 24 hours, or 15% of total blood volume in any 28-day period. This process may cause short-term discomfort and stress but is not expected to have long-term effects on the animal. In a limited number of cases, we will take bone marrow by sampling from cavity of a thigh bone. This route of sampling causes more pain and distress to the mice, so it will be conducted under anaesthesia and only when it is absolutely required (this technique is more efficient and requires a smaller number of cells injected). To ameliorate the pain 1) trained personnel only will perform the procedure, using aseptic techniques to minimise the duration and any trauma; 2) mice will be monitored for pain or distress (e.g., reduced movement, changes in grooming, hunched posture) after the procedure and provided with enriched food, water and an improved physical environment; 3) all mice sampled will receive analgesia (i.e. pain relief medication).

4. Blood Cancer Development:

In most of the cases, genetic alterations causing the cancer will be activated in mice only upon a specific treatment. As the timing of the activation is under our control, we are able to predict when the disease should begin and will perform enhanced monitoring with tailored intervention to minimise adverse effects occurring early.

Some mice will develop full blown blood cancer and/or develop gradual changes in blood cell numbers, which may lead to an enlarged spleen or liver. Occasionally, other tumours may appear. Signs of tumour growth, such as a swollen abdomen or lumps under the skin, and advanced disease symptoms like a hunched posture, raised hair, and reduced activity, will be closely watched for and will be humane endpoints.

5. Blood Cell Transplantation:

Blood cells will be transplanted into some mice (the animals receiving haematopoietic cells will hereafter be referred to as ‘recipient mice’), usually via the tail vein. This procedure may cause temporary discomfort and stress but is not expected to have long-term negative effects. In specific cases, abnormal blood cells may be injected into the cavity of a thigh bone (intra-osseus injection) to improve transplantation outcomes or modify experimental results. This route of injection causes more pain and distress to the mice, so it will be conducted under anaesthesia and only when it is absolutely required (this technique is more efficient and requires a smaller number of cells injected). To ameliorate the pain 1) trained personnel only will perform the procedure, using aseptic techniques to minimise the duration and any trauma; 2) mice will be monitored for pain or distress (e.g., reduced movement,

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changes in grooming, hunched posture) after the procedure and provided with enriched food, water and an improved physical environment; 3) recipient mice will receive pain relief medication (analgesia).

In some recipient mice receiving leukaemic cells, the cells may infiltrate (get into) the area around the nerves coming out of the spine. As these nerves are responsible for movement of the back legs, this can initially cause weakness (also known as paresis) and this sometimes can progress to full paralysis. Based on previous experience, this clinical sign may occur in some recipient mice between approximately 3 to 6 weeks post transplantation (this can vary slightly between different leukaemic cells). This symptom can be distinguished from a generalised reduction in their ability to move around; specifically, the front legs are unaffected, but both back legs are weak. These signs will represent the humane endpoints, and the mice will be culled when the humane endpoint is observed.

In such experiments where recipient animals receive leukaemic cells, to minimise the possibility of paralysis, drug treatment will be applied as early as the cells have engrafted (thus mimicking the human situation where patients are treated immediately post-diagnosis), so that the effects of new therapies can be observed prior to paralysis development. To reduce animal suffering, from the third week after transplantation we will include additional monitoring from the third week to catch any early sign as this is the period when animals would be more susceptible to this happening. In addition, in case this occurs, and animals cannot rear up to eat/drink, we will include floor food/wet mash/diet gel on the cage floor from week 3, so animals are not starved/dehydrated before they are found unwell, and the humane endpoint is applied.

Some recipient animals will demonstrate blood cancer-associated adverse effects such as discomfort, reduced mobility and decreased activity, that when combined with the administration of tumoricidal agents have the potential to cause lethargy and weight loss exceeding 20% (compared to a reference weight before cancer). Weight loss exceeding 20% will be our humane endpoint. Fatigue and severe weight loss is often seen in blood cancer patients before and during treatment for cancer. In order to monitor these effects and apply our humane endpoints we will employ rigorous monitoring and weighting schedules as well as provide mashed food (and or enriched diet) in order to ameliorate some of these symptoms.

6. Preconditioning for Transplantation:

Mice may undergo irradiation or other methods before transplantation. Irradiation is a standard method to prepare mice before transplantation. Following irradiation, the mice suffer from post-irradiation illness, where decreased food and water intake are main adverse effects and may lead to weight drop. The severity depends on the dose; low doses may result in mild weight loss (below 10%) and unchanged activity, with mice recovering within 7-10 days whereas high doses can cause more pronounced illness and weight loss of up to 15%. Recovery is also expected within 7-10 days. Partial body irradiation may require 20-30 minutes of anaesthesia but will not have long-term adverse effects.

Occasionally, mice may be pre-conditioned for transplant via the use of chemotherapy. This causes less damage to the bone marrow stromal cells (support cells that the blood cancer uses to help it survive and that can be important for drug resistance).

Mice will be weighed before irradiation/chemo and every other day to detect weight drop as a sign of ill-health post transplantation. Weighing of mice will continue until the weights are stabilised. To prevent the post-irradiation illness and weight drop, mice may be given enriched diet (mashed food, and/or

Dietgel) that may continue for at least 5 days post-irradiation. If weight loss exceeds the anticipated limit, mice will be provided with an enriched diet until their weight become stable.

Preconditioning by either method will be performed only once in a mouse's lifetime.

7. Surgeries:

Surgeries such as splenectomy or thymectomy (i.e., removal of the spleen or thymus, a method sometime used in clinic to alleviate the symptoms of leukaemia patients or to prevent the development of T-cell leukaemia, where the genetic alterations also predispose to this), will be performed under anaesthesia in germ-free conditions to prevent infection. Post-surgery, mice will be kept warm until fully recovered. Pain relief will be administered, and mice are expected to be fully active within two hours after surgery. Any signs of prolonged inactivity or pain will be humane endpoints.

8. Imaging:

Mice may undergo non-invasive imaging sessions lasting up to 45 minutes, with a maximum of nine sessions. Each session involves anaesthesia, and mice are expected to recover without long-term effects.

9. Anti- or Pro-Disease Treatments:

Treatments for up to 16 weeks to alter blood cancer progression will involve substances with known moderate adverse effects, such as reduced activity, decreased feeding, and weight loss of up to 15%, which may persist throughout the treatment period.

10. Physiological Treatments:

Physiological treatments, such as high-fat or nutrient-deficient diets, exercise, inflammation, infections, changes in gut bacteria, and light-dark cycle disruption, may result in mild changes in activity and weight, up to 10% of pre-treatment weight. These treatments will last up to 12 weeks.

11. Ageing related processes:

Ageing as a physiological process may cause mild effects like reduced activity, weight changes, hair loss or blindness. Aged mice will benefit from enhanced care and will be euthanised if they show signs of suffering. In rare cases, older mice may develop cancers or experience faster disease progression.

We will mimic ageing-related conditions through multiple approaches, including inducing mild inflammation, infection, disturbed dark-light cycle or dietary changes. These treatments may lead to weight drop as an adverse effect. The weights of mice will be checked at least twice weekly. To counteract the weight loss, mice will receive enriched diet before and throughout the treatment period to maintain their food intake.

All work will be conducted according to the principles set out in the Guidelines for the Welfare and Use of Animals in Cancer Research.

12. A very small number of animals (<1% in total) of mice will show swelling around the hocks. Any animals experiencing swelling around their hocks may be given an altered enrichment, pain relief

and/or anti-inflammatories in consultation with the Named Animal Care and Welfare Officer (NACWO) (i.e. the person who oversees the day-to-day care and welfare of animals used in our research) and/or the Named Veterinary Surgeon (NVS) (i.e. the vet responsible for animal health and welfare in our research facilities). On rare occasions (<3%) NSG mice (a mouse strain with a particularly weakened immune system) might display a somewhat hunched posture, that may get progressively worse with age (>5 months of age). When in isolation (i.e. without being accompanied by signs such as hampered movement, dehydration, weight loss (>10% from baseline i.e. a reference weight established and recorded before any regulated procedure takes place and/or before any clinical signs are expected)), or lethargy), this may not necessarily be an indication of illness/compromised welfare, but a naturally occurring phenotype due to the genetic background of this mouse strain (i.e. a natural and well recorded genetic predisposition of these mouse strains towards developing skeletal issues). On these occasions, advise will be sought by the NACWO/NVS, to exclude any other clinical symptoms.

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)?

We expect a mild severity (or less) in ~80% of mice, a moderate severity in ~15% of mice and a severe level of severity in ~5% of mice.

What will happen to animals used in this project?

- Killed
- Used in other projects
- Kept alive at a licensed establishment for non-regulated purposes or possible reuse

A retrospective assessment of these predicted harms will be due by 23 September 2031

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

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?

Human and mouse blood production processes and ageing are physiologically very similar, allowing us to use findings from mouse studies to understand human biology and validate research. The regulation of the blood system, especially the control of blood stem cells and their descendants, is not fully understood. Recreating the complex interactions between blood cells and their regulatory factors,

some of which are provided by other cells in the bone marrow environment (the so-called microenvironment) and that are abnormal in blood cancers, is challenging in a laboratory setting. Therefore, studying these processes in a living organism like mice provides a complete framework necessary for understanding both normal and diseased blood systems.

Technological advancements have made it possible to create mouse models with the same mutations found in patients with blood cancers. The mouse blood system is well-studied and provides a valuable resource for researching the development and function of normal and mutated blood cells. Differences between normal and diseased cells can be subtle, and animal studies can offer more precise testing conditions.

Mouse studies have a proven track record in advancing both basic and translational cancer research. Our work is increasingly focused on developing treatments, and it is crucial to test potential therapies in living animal models, before determining their suitability for humans.

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

In our project, we use a variety of methods to answer different questions. 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. Most of our work is done *in vitro* (outside the body, in a laboratory Petri dish or similar) where we grow immortalised cells (cells that do not die) as established cell lines. Sometimes we have used the cells from our mouse models to generate such cell lines and these can in some way replace experiments that we perform in mice. 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 microenvironment (supporting cells that nurture blood cell formation). These better mimic the situation in animals by providing some of the external support signals that we wish to study.

Human samples from both healthy individuals and patients are crucial for our project. The Project License Holder is the clinical lead and ethics holder for a Biobank of normal and malignant blood samples. This arrangement allows our institute to obtain fresh and archived samples of human bone marrow, blood and blood-supporting cells from both patients with similar blood cancers as well as normal individuals and individuals with pre-malignant disorders. These samples are used for cultures (growing cells in petri dishes) and other experimental analyses and can be used for transplantation experiments.

Using computer-based tools (bioinformatics) we analyse newly generated data as well as existing datasets and datasets published by other researchers to interpret already published data from animal, human and cell line studies. This helps us better and more efficiently answer our research questions and identify new directions for our studies. For example, these bioinformatic analyses will help to identify how mutant cells are likely to behave and what they are sensitive to therapeutically. These analyses can help to work out molecular mechanisms of how pre-malignant and malignant cells differ and can help predict conditions and treatments that might change their properties from growth to death. Throughout our research careers, this approach has proven to be an efficient way to gain knowledge, define areas for further study and to help answer some of the critical research questions without extensive animal experiments being performed.

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Why were they not suitable?

Due to the limited understanding of how the blood system is regulated in both mice and humans, it's not possible to fully recreate the natural conditions for culturing blood cells "in a dish." Once bone marrow or blood cells are removed from the body, they quickly lose their natural properties, making it difficult to study their biology accurately. Culture systems provide a simplified and artificial environment, allowing only specific questions to be studied without the full context.

In addition, the activity of drugs and the duration of their activity differ in a culture dish compared to their activity in an animal body. Therefore, studies in living animals enable scientists to draw conclusions that are more physiologically relevant. Therefore, findings from cultured cells must always be validated in living animals to confirm their significance.

A retrospective assessment of replacement will be due by 23 September 2031

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

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 are committed to minimising the number of animals that we use in our research. To ensure this, our strategy starts with careful planning of each experiment. We rely on our experience from previous licences, results from previous experiments and from predictive statistical tools (such as power calculations where significant differences between comparative groups are required) to answer our research questions. It is important to strike the right balance. Using too few animals can waste resources just as much as using too many, for example where under-powered experiments need to be repeated.

In our project, more than half of the animals will be used for breeding to generate mice with the specific genetic alterations required for our experiments. Some of our studies require animals to have two to four genetic changes, which can involve multiple breeding steps and result in a higher number of breeding animals. To make this process as efficient as possible, we optimise breeding strategies to obtain offspring that carry the required genetic profiles, which means that the least surplus mice will be generated. We also ensure only healthy mice are used for breeding to avoid producing animals unsuitable for experiments.

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

Study Planning:

- We use the PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines to carefully design studies and ensure quality.
- A detailed protocol is written for each experiment, including a clear description and the number of animals needed per group.
- We optimise experimental planning to ensure that repeat procedures are avoided, by collecting multiple types of data, and multiple tissues from a single procedure or sample.
- We perform well-designed pilot studies, to refine methods and determine the minimum required intervention, reducing unnecessary animal use and harm.
- If needed, we consult with expert statisticians to refine our plans.

Collaboration and Transparency:

- Plans are shared with the animal facility staff for smooth execution and to seek their input.
- Results will be published in open-access journals, following ARRIVE guidelines.
- Negative results may also be published to prevent others from unnecessarily repeating the same work.

Data Sharing:

- Genetic and sequencing data will be stored in the European Nucleotide Archive (ENA), making it accessible to researchers worldwide.

Reducing Animal Numbers:

- We use advanced methods like single-cell technologies (which allow the study of individual cells in detail rather than examining a group of cells collectively (through bulk analysis)) and bioinformatics (computational data analysis) to obtain more insights from the use of fewer animals.
- Cells from one animal are used for multiple experiments whenever possible.

By adopting these strategies and seeking professional advice when required, we aim to minimise animal use while ensuring high-quality research.

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

Optimal use of Existing Resources:

Whenever possible, we will obtain mice with the desired genetic changes from other researchers or facilities instead of creating new ones. This helps reduce the total number of mice required. However, if suitable models are unavailable and a specific variation or genetic background is essential, we will create new models using efficient techniques.

Efficient Breeding Strategies:

We frequently require mice with a combination of two or up to four genetic modifications. To generate mice with complex genetic profiles, we employ carefully planned breeding strategies to maximise the number of offspring with the desired genetic combinations. Whenever feasible, we use homozygous mice (with two identical genes) for mutations, provided these animals are healthy and fertile, to further streamline breeding.

Cryopreservation Benefits:

We regularly review mouse colonies to maintain only necessary lines. Mice without the required combinations of genetic alterations will be killed as surplus, as soon as they are genotyped. To avoid continuous breeding, lines not expected to be used for at least a year will be cryopreserved (frozen).

- Freezing reproductive material (sperm or embryos) safeguards against facility-wide risks, such as disease outbreaks, and reduces ongoing breeding needs.
- Best practices, including cryoprotective additives, are applied to improve recovery rates, even for historically challenging strains. This approach minimises the number of female mice required.
- Cryopreservation also preserves valuable mouse lines for future use in case of emergencies.

Collaborating and Data Sharing:

We will share our mouse lines and surplus mice with other researchers to prevent duplication of effort and reduce the need for additional breeding. Additionally, we will publish all results, including negative findings where appropriate, to avoid unnecessary repetition of experiments.

Using Relevant Controls to Minimise Variability:

To reduce the number of animals required for reliable results, we prioritise using littermates (siblings without genetic modifications) as the most appropriate controls. These controls share genetic material and environmental conditions with test animals, thereby minimising variability. For certain studies, we will use standard mice (e.g. C57BL/6) sourced from specialised facilities to ensure consistency, while avoiding unnecessary in-house breeding.

Use of Randomisation and Blinding:

To give confidence in results and reduce the likelihood of having to repeat studies, we will use randomisation and blinding. For example, in interventional studies, we will randomise animals to treatment vs placebo and keep operators (animal facility technicians and our own group technician) blinded wherever possible.

Maximising Data Output Per Mouse:

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- Whenever possible, we will collect multiple types of data from the same experimental animal to maximise the information obtained, while minimising inter-animal variability and total animal use.
- We will also review existing literature and collaborate with other researchers to refine experimental designs and avoid redundant studies prospectively.

Leveraging Advanced Animal Technology:

- Bioluminescence imaging (BLI): 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. As the development of disease can be investigated over time with BLI, 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, which is capable of emitting light when excited by the administration of an intra-peritoneal injection given to the mouse, prior to transplantation. 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
- We will also review existing literature and collaborate with other researchers to refine experimental designs and avoid redundant studies prospectively.

Leveraging Advanced Analytical Technology:

We will stay informed about new high-throughput and single-cell technologies that enhance data collection from specific and rare-cell populations, thus reducing the number of animals required. These cutting-edge tools allow for high-quality, comprehensive datasets from rare cell populations, improving the efficiency and impact of our research.

Studies of the Age-related Process:

Given the higher prevalence of blood cancers in older populations, our studies on ageing aim to understand how ageing-related factors influence blood systems and the initiation and development of blood cancers. We will optimise the use of aged mice by collecting various types of samples and sharing unused tissues through initiatives looking at ageing.

Balancing Efficiency and Scientific Rigor:

- While minimising animal numbers, we will ensure that sufficient mice are used to produce reliable and reproducible results.
- Long-term studies, such as those focussing on ageing may require additional animals to account for natural variability over time.

By following these strategies, we will conduct high-quality research while minimising the use of animals and adhering to ethical standards.

A retrospective assessment of reduction will be due by 23 September 2031

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The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

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.

In this project, we will use mice with specific genetic changes (mutations) to study why certain blood cancers develop and progress. These cancers occur when blood cells grow excessively without the normal control mechanisms.

There are a number of gene mutations linked to this group of cancers, and we have already created and studied several mouse models that carry these mutations. We have extensive experience working with these models, allowing us to carry out experiments effectively while minimising unnecessary harm to the animals.

In most mouse strains, the mutation in the mice is inactive, unless activated with specific treatments, meaning the mice remain healthy. Following activation, however, alterations in their blood system occur. Since we control when the mutation/mutations are turned on, and we know the effects that combinations of these mutations will have, we can accurately predict when the disease will start and take early steps to reduce any harmful effects. Most of the mutations we study cause slow-progressing diseases, which helps us further limit the impact on the animals' well-being.

Finally, much of our research uses material collected from mice after they are humanely killed, meaning the majority of our work does not involve causing suffering to the animals.

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

As detailed below, the unique biological complexity and genetic tools provided by mice are essential for achieving our study's objectives. Alternatives, such as frogs, fish, flies and worms lack the physiological relevance required for accurate and reproducible results.

1. Biological Relevance of the Model

The chosen species must match the biological characteristics required to answer the research question. For example:

- Mice are genetically similar to humans and have a well-characterized genome, making them indispensable for studying human diseases.
- Less sentient organisms, such as frogs, fish, flies and worms do not share the physiological, genetic or cellular similarities needed for translational research.
- For complex processes like immune response, neurobiology or cancer development, mice are the minimum viable model that accurately replicates human-like systems.
- Our lab is interested in blood cancers that typically present with a long latency and a discrete phase of pre-malignancy (i.e. the period between normal and cancerous state). This necessitates the use of adult, and invariably, aged mice to evaluate and dissect the biology of disease and any potential therapeutic interventions. This also precludes the utility of terminally anaesthetised animals for our work.

2. Availability of Genetic Tools

- Advanced genetic tools (e.g., transgenic and knockout technologies) have been pioneered and refined in mice, that are generally unavailable in less sentient species. These tools allow researchers to modify specific genes in a highly regulated manner and study their role in disease development and progression.
- Such precise genetic manipulation is critical for studying specific pathways and cannot be replaced by less sentient animals.

3. Physiological Complexity

- Certain studies require a vertebrate model to replicate complex organ systems, such as the cardiovascular, nervous or immune systems.
- Less sentient organisms often lack the necessary anatomical and physiological features to model these processes accurately. For example, flies and worms lack an adaptive immune system or the complexity of mammalian neurophysiology.

4. Ethical Considerations and the 3Rs

- The use of sentient animals is a last resort when less sentient organisms cannot yield reliable or relevant results.
- Replacement with alternative methods (e.g., computer simulations, cell cultures) or less sentient species is considered first, but they often fail to replicate the *in vivo* (inside the body) dynamics necessary for the research.
- By focussing on sentient animals only when required, we adhere to the 3Rs principle of ethical research.

5. Regulatory and Translational Requirements

- In many cases, regulatory agencies require testing on mammalian models to approve new therapies, drugs, or vaccines.
- Data derived from less sentient species are often insufficient for preclinical evaluation due to significant biological differences.

6. Advancing Human and Animal Health

- Studies conducted in mice often have a direct translational impact, contributing to the development of treatments for both humans and animals.
- Using an inappropriate model could lead to inconclusive or invalid results, delaying scientific progress and necessitating the use of more animals overall.

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

In this project, we will perform different types of experimental procedures. We refine procedures to minimise harms to animals. The highly trained staff at the animal facility offer significant practical assistance and general advice for refining protocols and post-procedure checks. These include:

1. Use of Anaesthesia and Analgesia

All surgical or invasive procedures will be conducted under appropriate anaesthesia to eliminate pain during the procedure. Post-operative analgesics will be administered to minimise discomfort.

2. Use of Non-Invasive and Minimally Invasive Techniques

- Whenever possible, we will use non-invasive technologies such as imaging to monitor biological processes.
- Blood, tissue, or other sample collection will be minimised by calculating the required volume and using advanced analytical methods to obtain data from smaller samples.

3. Improved Animal Handling

- Animals will be allowed to get used to handling and the research environment to reduce stress during procedures.
- Techniques such as tunnel handling (instead of tail handling for rodents) will be used to reduce stress levels in animals, particularly in aged animals.

4. Housing and Environmental Enrichment

- Housing conditions will meet or exceed regulatory standards, with stable temperature, humidity, light cycles, and noise control. To satisfy the natural social instinct of mice, animals will be housed in compatible groups to reduce stress and isolation, unless welfare issues arise (e.g. individual housing due to aggressive behaviour).

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- Animals will be provided with enrichment such as nesting material, shelters and social housing to promote natural behaviours and reduce stress.

5. Skilled Personnel and Continuous Training

All procedures will be conducted efficiently by skilled and well-trained personnel, ensuring they are completed in the shortest time possible to minimise stress and discomfort for the animals. Regular training and updates on best practices for animal care will be conducted.

6. Monitoring and Intervention

- Animals will be closely monitored for any signs of pain, distress or illness. If such signs are observed, intervention protocols, including administering additional analgesia or implementing humane endpoints, will be promptly applied.

- The well-being of ageing animals will be carefully monitored through regular checks of their condition. Standard checks will include observations of their activity, skin and fur condition, teeth, posture and weight. The observations will be cross-checked with previous reports to detect any signs of deterioration. When deterioration is observed, appropriate countermeasures will be taken, which may include supportive diet, additional cage enrichment or specific treatments (e.g. medicaments applied to the skin) upon consultation with the Named Veterinary Surgeon and Named Animal Care and Welfare Officer.

- Detailed welfare scoring systems will be used to objectively assess animal well-being and adjust care or procedures as needed for the studies.

- Any animals experiencing swelling around their hocks may be given an altered enrichment, pain relief and/or anti-inflammatories in consultation with the NACWO and/or NVS.

7. Humane Endpoints

Humane endpoints will be clearly defined and strictly adhered to, ensuring unnecessary suffering is avoided. Animals will be humanely killed if predetermined welfare thresholds are met.

8. Refined Experimental Techniques and colony management

- Employing single-cell or high-throughput technologies will reduce the number of animals required while maximizing data output.

- Micro-sampling and refined surgical tools will be employed to reduce procedural invasiveness and recovery time. Ear notching not only provide a method for animal identification but is also used for determine their genetic profiles, all of this information is stored in a well-designed web-based system to enable easy access and efficient management of mouse colonies.

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

https://www.nc3rs.org.uk is our reference for best practice in animal research. Various aspects of working with animals such as handling, anaesthesia, analgesia, blood sampling, euthanasia and welfare assessment are covered there under the specific section as in the link:

https://www.nc3rs.org.uk/topic-specific-resources-0

Equally, we will refer to http://www.lasa.co.uk/ where under the tab “Current Publications”, we can find "Guiding Principles" for different aspects of working with animals. Particularly rich information can be found there regarding the principles for aseptic surgery:
http://lasa.co.uk/PDF/LASA_Guiding_Principles_Aseptic_Surgery_2010.2.pdf

To determine the number of animals matching our experimental needs, we will follow the assessment framework available from the Home Office under the link:

https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/773553/GAA_Framework_Oct_18.pdf

For best practice, all experiments will be planned in agreement with PREPARE guidelines (https://norecopa.no/prepare) and we will follow the ARRIVE guidelines (https://norecopa.no/3r-guide/arrive-guidelines) to report our animal research.

All PIL holders working under this PPL will be strongly encouraged to thoroughly study and implement these resources in their experiments.

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

We stay updated with advancements in the 3Rs (Replacement, Reduction, and Refinement) by regularly reviewing newsletters provided by our establishment’s Named Information Officer. We will check the NC3Rs website (https://nc3rs.org.uk/) for updates and follow publications on the LASA forum (https://www.lasa.co.uk/current_publications/). Additionally, we use the establishment's 3Rs Search Tool to explore relevant resources.

All team members are encouraged to familiarise themselves with these materials. Any important updates related to the care and welfare of our mice will be shared during lab meetings, in mouse activity-focussed meetings or via email lists. We strive to set a good example for our colleagues and maintain a close working relationship with the animal facility staff to ensure the best possible care for the animals under our supervision.

A retrospective assessment of refinement will be due by 23 September 2031

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?