



Home Office

NON-TECHNICAL SUMMARY

Haematological development and functional characterisation in tumour models

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

Immunology, Cancer

Retrospective assessment

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

Objectives and benefits

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

What's the aim of this project?

We wish to investigate how parts of a cell's genetic instructions (found on DNA) can influence the development and function of the cells that circulate in the blood such as immune cells, red blood cells and platelets. The cells are very important for normal health and in fighting infections and it is now becoming evident that they can be used to target cancer. When their development or function goes wrong this can result in an inability of the body to generate certain cells and defects in fighting infections (immunodeficiency) or a lack of control which results in the body attacking itself (autoimmune conditions). Through large human sequencing studies, such as 100,000 genomes run with the NHS, we are beginning to identify more parts of DNA that could be responsible for these conditions and be important for development and function of these cells. However, even if we identify these regions from human studies we need to be able to confirm that these are the cause and to then understand how they regulate these processes.

In this work we plan to help identify these parts of DNA and gain insight into how they can regulate the development and function of blood cells. One particular area of interest we have is how these cells function towards cancer cells and if we can manipulate this to develop new therapies. We will investigate this using a model of primary tumour growth and also a model of when tumours spread to other places around the body, so called secondary tumours.

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.

What are the potential benefits that will derive from this project?

One benefit will be to provide evidence to confirm or eliminate particular parts of DNA as being causal for human diseases such as immunodeficiency or autoimmune. This will help in designing screening tools to diagnose these conditions in the clinic and by understanding how a particular alteration can cause a disease could eventually aid in the development of new therapies. For other scientists these studies will provide information regarding the function of some of these regions within blood cells which will allow for additional follow up studies. We will also generate large datasets and mouse lines that other scientists can use rather than generate their own. Investigating the interaction between blood cells and cancer might lead to the identification of new ways to help a patient's own cells fight off cancer cells which could be developed into new cancer therapies. This could be in making ways to make a patient's cells survive and function better within a tumour, identifying ways to boost their function towards killing cancer cells or finding new ways in preventing them from being converted into cells that dampen the activity of other blood cells.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

We plan to use mice in this study and up to 72,700 over 5 years.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.**In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?**

As we will be generating genetically altered mice (where part of the DNA has been affected) approximately one third of the mice we will use is in breeding to be able to perform our experiments. These mice will have small pieces of tissue taken to allow us identify them and determine if they carry the mutation we desire. On occasion we may take small blood samples from them (typically the tail) and these are classified as mild severity. A small number of mice will be used to generate materials such as sperm and embryo that allow us to store these genetically altered lines for many years at a low temperature. In another one third we will analyse the mice that we have generated and we shall take blood samples, and after humane killing, examine tissue samples. Some of these mice will have the function of their immune system checked, this will be achieved by injecting the mice with substances such as vaccines or cells, prior to analysis of their blood and tissues after humane killing. As these blood cells move around the body we may alter this either by injection or placing substances in the food or drinking water. For some of these mice we may collect larger blood samples when the mice are deeply anaesthetised prior to increasing the anaesthesia administered and humanely kill and/or flush their body with saline to remove all the circulating blood from tissues to enable us to study the cells within tissues as a method of humane killing. All these procedures are done under deep anaesthesia and are classified as mild severity. After collection of blood samples from the tail on rare occasions mice may develop an infection or bad scarring, in this case they will be humanely killed. The final third of mice will undergo procedures that are considered to be of moderate severity. Some of the mice will have their blood cells replaced by performing a bone marrow transplantation, here a host mouse is exposed to a source of radioactivity which kills all their blood cells, which we then replace with cells harvested from another mouse. These mice are briefly susceptible to infection while their blood cells recover and so we give them an enhanced diet to assist recovery and ensure they are healthy prior to this procedure. We will collect blood samples from these mice to monitor the recovery of the blood cells and analyse tissues after they are humanely killed. On very rare occasions they can develop sickness related to the procedure and will be humanely killed if their condition does not improve. We know some of the factors that can give rise to this sickness and mice destined for this procedure are subjected to extra health checks. The remaining mice will be used in our cancer studies and will be injected with cancer cells to their side to generate a small lump or into their tail vein to give rise to small masses in the lungs and/or liver of the mice. Some of these mice will be briefly anaesthetised so that we can look at the cancer cells via imaging methods and we can track how they develop. The small lumps on the side of the mice are measured at least twice per week to track their size and mice are humanely killed when they reach 1.2cm². A subset of these mice will be used in our therapy studies and we will investigate ways to target cancer cells. This could be achieved via administration of purified mouse or human immune cells (which we may manipulate in the laboratory) or substances to boost the function of immune cells for example an anti-cancer vaccine or treatments that are currently used to treat patients. When the mice reach the endpoint of the study, defined by a humane endpoint such as tumour size or timepoint, they will be humanely killed prior to tissue collection. Some mice could have small blood samples collected while they are alive so that we can track the effect of a treatment or larger volumes collected after they are humanely killed. Mice typically do not show any altered behaviour when administered cancer cells, however on some occasions they may not have control of the cancer cell growth and while we can easily monitor those where the tumours are on the side of the mouse for the

lungs this would be evidenced by rapid breathing and the mice humanely killed. We select the cancer cells to be administered to be the most suitable for our experiment which give rise to masses that are well tolerated. Very rarely the mice may scratch at their lumps causing the skin to be broken and if this is observed the mice are humanely killed. Some of the immune system treatments, as they are designed to provoke the immune system, can give rise to symptoms such as increased temperature and diarrhoea. This will be closely monitored and when it exceeds certain thresholds the affected mice will be humanely killed. The models and experimental protocols that we are using are well established and designed to cause the least pain, suffering and distress.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

The cells of the blood system and their interaction with tumours is very complex and requires the interaction with other cell types for which it is not possible to use a non-animal alternative. We will use existing data sources rather than duplicating where these exist and also harvest additional tissues for use in alternative lab-based experiments where possible. Should new lab-based or computer based models exist which generate comparable data these will be adopted.

Reduction

Explain how you will assure the use of minimum numbers of animals.

When a suitable mouse line exists we will import the line rather than generate a new line. All lines that we generate in this study will be archived in a suitable international repository to be used by other interested researchers. The gene expression data that forms part of the identification of parts of DNA that we are interested in will be shared in open access repositories to enable other researchers to use our data rather than generate additional data sets. We will use various statistical approaches to determine the minimum number of animals to use in an experiment and where possible combine experiments. We will also harvest additional tissues from mice at the end of experiments to use in laboratory-based assays to reduce numbers needed. Proper design of the experiments and controlling for sources of variation such as the age and sex of the animals will also help increase the robustness of the experiment and result in an overall reduction in animals needed. When publishing our data we will follow the ARRIVE guidelines to ensure comprehensive reporting and will release all data including where we do not find any alterations as this can be as informative to prevent assays being repeated in other laboratories.

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Mice represent the best choice of animal for these studies given the similarity between the human and murine immune system. Furthermore, they are very amenable to genetic manipulation allowing us to perform the studies we plan. The use of mice in cancer studies is well established and the models we plan to use are considered 'gold standard' and are used in the development of therapies that are now used in the clinic and resulting in good responses in some patients.

In all our experimental work we will minimise the number and severity of procedures applied to the mice, this could be via the selection of the substances that we administer causing the least number of side effects, administering substances together if possible, or switching to oral administration in diet/drinking water to minimise injections. We will use cancer cells that are fully characterised and will investigate alternative methods to monitor the growth of the cancer cells in the mice to minimise stress from handling. When we are performing experiments requiring mice to be anaesthetised for a period of time we will use agents that allow for a rapid recovery and that have minimal build up. Mice will also be kept warm while asleep and if they are asleep for a while administered fluids to help speed up recovery and reduce dehydration. When we perform bone marrow transplantation mice are thoroughly health checked to eliminate those who may not tolerate the process as well. They are also provided with wet mash to aid recovery and antibiotics to reduce the chance of infection. We will use published data to define the best agents to use in our studies, picking those that are the most clinically relevant where they exist.

The use of a sophisticated mouse tracking system allows accurate tracking of all health concerns associated with the mice to be used in this study and to enable rapid investigation where they occur at a higher than expected incidence. All people who work with the mice in this study are thoroughly trained and continuously assessed for their ability to perform these procedures, with procedures refined following advice from the vet/NACWO or other international guidance.