Home Office

NON-TECHNICAL SUMMARY

Interaction between the immune system and tumours

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

cancer, immune cells, gene, immunotherapy, function

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

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?

To understand how genes (which are the key instructions inside the body) within the cells of the immune system (that normally helps protect the body against infections) can effect the way in which these immune cells interact with cancer cells. This can result in an alteration to tumour growth or spread or the direct killing of cancer cells. This will also include studies on how particular aspects of cancer cells can affect the function of immune cells within 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.

Why is it important to undertake this work?

It is now predicted that 1 in 2 people will develop cancer in their lifetime and despite advances with new treatments they do not work for everyone. By providing further understanding how the immune system can detect tumours and eliminate cancer cells it will provide new avenues for treatments.

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

We will generate a list of genes that can affect the immune cells and their response/survival within the tumour microenvironment, this may also include genes that do not have any effect which is also important to define. This will be in the format of publications in peer-reviewed scientific journals as well as presentations at local, national and international scientific meetings. All data will also be released to open data sources. Depending on the availability of reagents we may also identify potential drugs that could go into clinical development.

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

Short term:

Other research scientists will benefit from lists of genes that can affect immune cells and their response to tumours as well as genes that have no effect. They will also benefit from understanding if these genes influence general immune cell biology or act specifically within the tumour microenvironment

For clinical colleagues the understanding of gene function within the immune system will add further candidate genes and additional information for the identification of causal genes for human immune disorders.

Long term:

Other research scientists will benefit from new understanding for the effect of the tumour microenvironment on immune cell function and the genes which can regulate this.

To the wider pharmaceutical industry and patients this could lead into the development of new anti-cancer therapies.

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

We collaborate with several academic researchers in related fields sharing data, methods and reagents. More broadly through our funding we have strong links to several pharmaceutical companies which allows early triage and feedback on the data and direction to help ensure the most promising targets are followed up. Via open access data release sites all data will be released and if possible negative results will be published to allow others to benefit from the knowledge.

Species and numbers of animals expected to be used

- Mice: 5770

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.

We are using mice and typically adult mice because we need a fully developed immune system for our studies which occurs after about 6 weeks of age. The mouse immune system is highly similar to that of humans and allows us to translate our findings. We can also access key immune tissues as the blood only contains certain cell types and the immune response can be restricted to particular areas of the body. There is also a large range of high quality reagents which ensures that the data is reproducible and robust. There are very good models of a variety of cancers for use in mouse and these have been successfully used to identify new treatments which cancer patients are using.

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

We estimate that 55% of mice will be used for the generating mice to be used in our experiments or for the collection of tissues. When they are used in breeding they will be mated with other mice carrying the required modification to their genetic material and we will sometimes have to take a small piece of tissue (normally from the ear) in order to check that the offspring contain this information. For mice used in breeding they will typically be kept up to a year of age. For those mice that are used for tissue collection this may include taking small amounts of blood while they are alive to check the presence of particular cells or after killing to take particular immune organs in order to isolate cells. These cells may be studied in the laboratory and some will be transferred into other mice to use in experiments. The mice used for the collection of tissues are typically aged up to about 6 months although sometimes we may investigate the effect of age by keeping them up to just over 1 year of age.

We expect about 5% of the mice to be given other immune cells by injection into a vein or a body cavity in the abdomen. They will then have small amounts of blood collected so we can track what the cells are doing. In some cases we may give the mice some compounds by injection or in their food or water which we predict will affect the cells in order to determine the role that they have.

About 40% of mice will be administered cancer cells with a small injection to their side or a vein which will either develop as a lump or within the body. Some of these mice will have small amounts of blood collected from peripheral blood vessels while they are awake so we can monitor the response by tracking the number and type of cells present. In some cases we may inject the mice with additional immune cells into a vein or into a body cavity in the abdomen to be able to determine if the alteration we have made makes the cells better at getting into the tumour or can respond better which would make the tumour get smaller. We may also give the mice some treatments similar to those that are used in hospital to discover if what we have identified can help these treatments work better again monitoring the tumour size. These treatments may be administered by injections into a vein or into a body cavity in the abdomen or where possible in the food or water. In some cases we will image the mice by briefly anaesthetising the mice and injecting them if needed (via a vein or into a body cavity in the abdomen) with a chemical that glows in the cells that we have previously given to the mice, this allows us to track where the cells are within the body over time. At the end of the experiments the mice are killed and tissues are collected in order to complete other studies on the material. The mice are typically kept for 2-3 months on these experiments but a small number will be kept for longer (up to 6 months) if we are investigating if the tumour can return or the mice are 'cured'.

Around 2% of mice will be used in the generation or recovery of genetically altered lines where they may have a small surgical procedure performed in order to transplant embryos or to make them infertile. Alternatively they will be given drugs via an injection into the skin or a body cavity in the abdomen, to increase the numbers of eggs that they produce to allow us to cryopreserve the genetic alteration similar to the process of in vitro fertilisation. This allows us to freeze embryos and sperm for the future and share with other researchers around the world, without needing to constantly breed the animals or transport them to other countries.

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

The genetic alterations that some of the mice in the project carry are not expected to have any effect on the mice and they would live a normal life when housed in our facility. As the study can be affected by the condition of the mice where there is evidence of fighting the mice could be solo-housed or additional enrichment added to the cage in order to reduce the impact.

After administering the cancer cells the mice are typically not affected by the developing lump on their side or tumours within their bodies which normally occurs in the lungs. On rare occasions if the mice have a severe defect with the immune system the cancer cells will grow very fast and the lump can become irritated which can lead to the skin surface breaking and this is a humane end point and the mice would be killed. Alternatively if the mice have cancer cells in their body they could appear to struggle with breathing or have an enlarged body in both of these cases the mice would be killed.

When we administer the treatments the mice do not typically show any side effects, if the response is good they can show a little sickness behaviour similar to a mild cold that normally resolves within a day. Occasionally if the effect is too strong or there is an unexpected interaction with the cancer cells or

the immune cells they can develop signs of autoimmune disease which typically presents with diarrhoea and other digestive issues, in this case the mice would be killed.

Sometimes if we give substances to the mice in their food or drinking water this may taste different and so they can avoid drinking/eating. Any mice that are put on a modified diet/water are weighed before starting and at regular intervals to check that there are no problems with weight loss. We can also add some sweetener or other substances to mask the flavour.

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)?

Mice

Mild 62%

Moderate 37%

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

- Killed
- Kept alive
- 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 immune system is very complicated as it is spread out across the body with specialised regions which is not possible to model in a dish.

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

We considered a variety of laboratory based models such as cancer cell and immune cell cultures and also tumour organoids.

Why were they not suitable?

The simple co-culture of immune and cancer cells does not allow us to fully model the complexity of the immune system where a key aspect is the specialised microenvironment that exists within a tumour and also the process of migration of various cells around the body. Tumour organoids do have more of

a microenvironment, however, they need many inhibitors and growth factors to be maintained which often effects the function of the immune cells. Also it is not possible to address how immune cells can enter or are prevented from entering the tumour microenvironment in a tumour organoid and also address questions regarding the role of other specialised immune sites that are only formed in a living animal with an immune system that is similar to that of humans.

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?

Pilot studies are used to establish the correct dose of reagents/cells, timelines, and to generate baseline data for new experiments. We have refined many of the experimental procedures we are following to ensure that they are robust and reproducible while enabling the detection of effects using the minimal number of animals.

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

All downstream analysis on tissues is performed blinded and where possible mice are randomised to treatment groups across cages/genotypes, this allows for more robust measurements of effects. This together with optimised and refined experimental procedures ensures studies are performed in the most robust and reproducible manner to generate the data with the minimum number of animals. This can be achieved by pooling the data from smaller cohorts over time to reduce batch/cage effects and using specialised statistical methods that enable the analysis of this type of experiment.

Appropriate controls are used (this maybe WT animals or mice injected with control substance/cells).

Online resources such as the NC3R's experimental design assistant and the PREPARE guidelines will be consulted.

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

By standardising the experimental protocols this allows the analysis of data to be achieved overtime and the pooling of data from smaller cohorts. This is especially useful in the case of genetically altered lines where there maybe an effect of survival and/or fertility to allow the breeding to be kept to a minimum level as we do no need such large numbers of breeding mice to generate the number of animals to go into our experiments. Also with standardised experiments and the completion of pilots we are confident that the outcomes are robust and reproducible. Animals that enter experimental

procedures are health checked as we have observed that fight wounds greatly impact on the outcomes and these mice are excluded.

Where possible we share tissues with other groups and we can store tissues from animals for future ex vivo studies. We also keep up to date with resources and guidelines such as PREPARE and the NC3Rs experimental design assistant.

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.

Many of the animals used will be genetically altered this could be to add a gene or reporter or to delete a gene or set of genes depending on what is required for the study in question. None of the genetically altered animals have any clinical signs under normal conditions.

For some of the animals we want them to develop tumours which we do by administering cancer cells to their side or in the blood stream, here we follow current national and international guidelines and use the smallest needle and volume to minimise the effect on the animals. The resulting tumours will grow as a small lump on their side or within the lungs and the animals do not typically exhibit any clinical signs. We have monitoring in place to identify animals that are performing in an unexpected manner and for the tumours on the side they are regularly measured and checked to ensure they do not cause any discomfort. There is a maximum size that the tumours can reach before the animal is killed and as the data is routinely reviewed during the experiment if the scientific end point has been achieved we will stop the experiment early.

We also need to administer immune cells or substances that can affect the immune system this is done by the least invasive route possible for the substance and following current national and international guidelines using the smallest needle and volume to minimise the effect. When more than one substance is to be administered these are combined where possible to reduce the overall number of injections and the site of injection is alternated. We will also administer substances in the diet/drinking water where possible.

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

We considered using lower animals (such as zebrafish) but the translational potential of these in the context of immune-tumour interactions is not yet known and thus mice are considered the gold standard for the development of new therapies in this field. As we are studying the role of the immune system we need to have an intact immune system that is comparable to that of humans and for this reason mice are the best model. We need to allow time for tumours to develop to be able to assess the effect of genes on the ability of the immune system to control the tumours and so these experiments

can take weeks or months not enabling the use of terminally anesthetised animals. As the immune system isn't fully formed at birth we need to use adult animals for our experimental work.

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

We have a lot of experience with the proposed models and have refined and developed the clinical monitoring, however, we do always keep up to date with new developments and will look to implement them. Any animals that are to enter an experimental protocol which requires several interventions (such as administration of substances, tumour monitoring etc) will be habituated to handling prior to the experimental procedure starting.

We will constantly research new methods that will result in fewer interventions being applied to the mice or to enable the administration of substances via a less invasive route (such as in the diet/drinking water) and these will be implemented.

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

With regard to preparing our work for publication we follow the ARRIVE and PREPARE guidelines.

We follow the American Association for Accreditation of Laboratory Animal Care and the Laboratory Animal Science Association guidelines for administering substances and withdrawal of blood. For our tumour work we follow the guidelines of the National Cancer Research Institute.

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

We subscribe to the NC3Rs email updates and also receive regular updates from the named people at our establishment. There are also several online sources of advice such as the NC3Rs website and Norecopa.