Home Office

NON-TECHNICAL SUMMARY

# Germinal Centre B cells in physiology and pathology

**Project duration**

5 years 0 months

**Project purpose**

- (a) Basic research

**Key words**

Immunity, Cancer, Lymphoma, Infection, Autoimmunity

## 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?**

Our immune system is composed of a network of cells forming the innate immune system and adaptive immune, that together protect our body from infection by "foreign" invaders (pathogens) such as bacteria, viruses, fungi and parasites. In addition to these properties, the immune system functions as

an important barrier to cancer formation and progression. However, the cells composing the immune system are themselves susceptible to become cancer cells being at the origin of haematological cancers, for example lymphoma mostly derived from cells called B-lymphocytes.

Our broad aim is to investigate the processes and the molecular mechanisms that predispose normal B-lymphocytes to become cancer cells. More specifically the objectives of our work is to i) identify and study cellular subpopulations within healthy B-lymphocytes that due to their physiology are at a high risk of becoming cancer cells, so-called “cells of origin in cancer”; ii) investigate causative genetic aberrations that are key in the progressive process of how a healthy B-lymphocyte becomes a cancer cell; iii) how do cancers derived from B-lymphocyte evade the immune system; iv) what are the mechanisms of resistance to therapy of these cancers; v) what is the role of infection in the formation and progression of B-lymphocyte derived cancers.

To achieve these objectives the proposed work uses genetically altered mouse models of pre-clinical relevance. These models are absolutely required to fully understand the cancer initiation and progression, at least in part due to the complex nature of the interaction of cells of the immune system between themselves and with those present in their microenvironment.

**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?**

The expected benefits of the work can be summarised as follows:
1. We will gather a deeper knowledge of the pathways, genes and immune conditions key for cancer initiation and progression; identify causal mutations in cancer; and will integrate this knowledge with the role of immune system in cancer formation and progression.
2. The information gathered in these studies should allow a better definition of the risk of cancer formation, progression and relapse and assist the identification of biomarkers and development of novel and effective therapies, relevant for the design of future cancer treatments by the pharmaceutical industry.
3. The cancer models developed in these studies will be of pre-clinical importance and of value to other scientists for the development and testing of anti-cancer therapeutics, including therapies aiming to stimulate our own immune system to recognise and eliminate cancer 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?**

Mice are the model we will be using in this project and we predict that a maximum of 10,000 animals will be used per year over the 5 year term of the project.

# 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?**

The experiment proposed to be performed during this project will be of mild and moderate severity. Irrespective of the procedure all animals will be monitored closely and will be humanely killed if unexpected ill health occurs, if severity limits are approached or if scientific objectives have been attained.

We will assess phenotypes in mice under steady conditions and upon immune challenge. The immune challenge includes one of the following situations: inducing an immune response by immunisation or by infection with a live pathogen. For the majority of cases no significant adverse effects are expected and the most likely common reaction will be weight loss and fever leading to signs of distress. On occasion, specific viral infections may induce a weight loss and other health signs that may reach moderate severity. However, these mice are expected to make a full recovery upon resolution of the infection.If any of these adverse effects are not resolved the mice will be removed from the study and humanely killed.

An expected phenotype of moderate severity is the development of cancer. The health status of these mice will be monitored closely for signs of persistent discomfort and to ensure the tumour size does not exceed the recommended guidelines. On occasion, to investigate the progression of the cancer and resistance to therapy we will have to perform surgery of tumour bearing mice to extract a biopsy of the cancer. These experiments are required to perform investigations that analyse the same tumour in the same mouse as this is the only way possible to determine with certainty the genetic mechanisms of resistance to therapy. Surgeries will be performed by experienced staff and after surgery animals will be monitored for clear signs of recovery from the surgical procedure. In a situation where animals do not show such signs of health recovery, they will be removed from the study and humanely killed.

# Replacement

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

While valuable studies of human cancer are performed using tumour material, the mechanistic understanding of cancer pathogenesis requires the use of living animals. In particular the development and function of the immune system involves many different cell types interacting in a dynamic three-dimensional environment. Similarly, cancer development and spread involves a plethora of interactions between cancer cells and their surrounding cells, governed by multiple signals originating from both their immediate neighbours and from distant tissues.

The study of cells in culture provides us with cues on the mechanisms of cellular processes in a simple and defined context, which allows the establishment of hypothesis of the function of cells in an intact animal. However, these systems do not recapitulate the complex cellular interactions described above. Therefore the use of animals is essential.

# Reduction

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

Before experiments are performed, we will collect evidence from current literature, analyse existing datasets both mouse and human that have analysed normal cellular population as well as cancer conditions. These analyses will be, whenever possible, confirmed using primary patient material. We will also perform studies in vitro using established cancer cell lines and mouse primary non-transformed cells. These studies will precede and guide the generation of relevant transgenic mouse models.

The breeding of transgenic animals will be reduced through collaborative access to strains and by avoiding overbreeding. For example, genetically altered mouse lines used only sporadically will either be maintained at low levels, or frozen whenever practicable, and/or maintained in collaboration with other licences to minimise redundant breeding.

The proposed experimental designs and methods of analysis will be discussed with members of the laboratory, and those of our collaborators, and we will seek additional advice from the statisticians employed by our Institute. We will perform pilot experiments in which a small number of animals per group are used for genotype comparisons. Depending on the results obtained from pilot studies we will then proceed to perform larger cohort studies to determine if the observed difference is statistically significant.

Further we will use whenever possible modified bone marrow cells for the reconstitution of the immune system in host animals, which permits the increase of sample measurements together with the reduction of the breeding of genetically altered animals. This approach also allows bypassing complex genetic crosses aiming to identify intrinsic versus extrinsic phenotypes.

When performing tumour studies we will, when possible, follow up tumour development using whole body scanning as it allows a reduction of the number of animals used. This strategy goes along with the concept of not using more animals than the ones essentially needed to obtain informative and statistically significant data, and to maximise the data obtained from each animal, e.g. by studying the effect of mutations in multiple cell types.

# 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.**

The mouse is one of the model organisms which most closely resembles humans and its genes are mostly functionally conserved. Of particular relevance to answer the scientific questions in this project mice have genes of the immune system not represented in other non-protected animal model organisms like the nematode worm and fruit fly.

Mice can be experimentally genetically altered, there is already extensive literature concerning the topics of our investigation, and our own studies can be enhanced by combination with many complementary models developed by others in the field. We will strive to generate transgenic mice in which mutations are induced specifically and conditionally in the cell population of interest, using for the effect e.g. Cre-LoxP conditional alleles or alleles, which function can be activated or terminated through the use of Tet-On and Tet-Off systems. These mice should not display a phenotype until the mutation in the candidate gene is induced.

In all our experiments we will set humane endpoints and write an experimental protocol, which will include details of possible adverse effects. For example, when administering substances or cells to animals, the route used for delivery will be such as to achieve “best practice”, that is to minimise or avoid adverse effects, while minimising the number of animals used, and maximizing the quality and applicability of results. For that reason we propose in this project licence a variety of routes of administration of substances and cells to achieve the scientific objectives, while minimizing the waste of animal’s lives.