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NON-TECHNICAL SUMMARY

Mechanisms of cancer development

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, heterogeneity, stem cells, epigenetic

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?

The overall aim of the study is to understand what makes cells behave in an abnormal manner in cancer, with the ultimate goal of using this knowledge to develop novel strategies to treat the disease. Cancer starts because cells "forget" what they are and what they should do in the organ where they

reside. This “amnesia” is accompanied by acquisition of novel features that make cells capable of dividing without control, hide from the immune system, create an environment in which they can keep growing and spread to other organs. At the heart of this cellular transformation there are both genetic changes - mutations that alter the DNA sequence - and non-genetic alterations that make cells use their DNA inappropriately. We study the non-genetic processes that drive cancer development, and try to understand how we can interfere with these processes to benefit patients. While mutations cannot be erased, recently developed drugs allow us to change non-genetic alterations of cancer cells, providing new hope for more effective treatments.

By focusing on non-genetic process that make cancer cells misuse their genes, we are interested in addressing two major issues:

1) Understanding how loss of cell memory favours cancer initiation. Numerous cancer-driving mutations have been found in normal tissues that do not contain any cancer, indicating that, by themselves, mutations are not enough to start a tumour. By understanding what additional non-genetic events are needed to initiate the disease, we hope to generate knowledge that will help early cancer detection.

2) Understanding what determines which cancer cells are truly immortal and can drive disease progression. Tumours are made up of highly diverse cells. Even neighbouring cells within a tumour may have distinct shapes and behave differently. Most importantly, not all cancer cells can divide in same way and in most cancers only a subset of cells is truly immortal. These cells, known as cancer stem cells (CSCs), are those that make cancers grow and invade healthy organs. Often, these cells are resistant to conventional chemotherapy and are responsible for disease relapse, which in many cases leads to patient death. Understanding how these cells function and what makes them different from the chemotherapy-sensitive cancer cells will allow us to design more effective strategies to treat the disease. Very little is known about CSC characteristics and we aim at casting light on the non-genetic processes that regulate their function within a tumour.

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 primary potential benefit of the proposed study is to increase our knowledge of how a tumour grows and identify new ways in which cancer cells can be killed or made harmless. The information is likely to be directly relevant for studies focused on designing novel anti-cancer treatments. Because non-genetic processes are intrinsically reversible, and several drugs interfering with this processes are available, the path to developing novel treatments may be relatively quick. In addition, our findings may assist oncologists in cancer diagnosis and prognosis.

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?

For our study we will use mice, the best model system to study cancer biology. Based on the work carried out in the past 5 years by my laboratory under our previous licence, the number of mice

expected to be used over the 5-year period will be approximately 30,000.

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?

In this project we will mainly create tumours in mice where specific conditions are altered, trying to understand how CSCs are formed and make tumours grow.. To achieve these aims, we will use a variety of well-established experimental techniques, including breeding of mice which will develop tumours spontaneously, induction of tumour growth by injecting tumour cells or exposing animals to chemical or biological agents. In most cases (~70%), tumours will be superficial and will not affect organ function, impacting only minimally on the animal's overall condition. On rare occasions only, minor surgical procedures, such as skin biopsies or resection of small tumours will be carried out. Non-invasive imaging techniques, similar to those performed on patients, will also be performed under general anaesthesia to monitor tumour growth. At the end of the experiment, animals will be humanely killed and tumours harvested.
Overall, we expect mild or moderate adverse effects associated with the protocols used in this project. To be able to achieve our scientific objective, about 50% of the animals used will need to grow tumours to a size larger than that recommended by the NCRI guidelines. This is necessary because one of the main purposes is to induce tumour formation with the goal of producing, in a controlled fashion, high numbers of CSCs which will be then studied after tumour collection post-mortem. To allow proper diversification of cancer cells and ensure that enough cells will be available for analysis after tumour removal, it is necessary that tumours grow to 2 cm in diameter over a 2-month period. However, we have previously seen that such large tumours cause no more than moderate pain or distress to animals, since they are typically very superficial and do not affect organ function. Due to superficial nature of the induced tumours, up to 30-40% of the animals may develop small ulcerations. We have extensive previous experience with similar experiments and have seen that small ulcerations most of the times do not affect animals' well-being. The use of suitable analgesia will help minimizing animal suffering. To be able to achieve our scientific goal reducing the number of animals needed to obtain statistically significant results, animals developing ulcerated tumours will not be euthanized. For most of the animals we expect only mild adverse effects, but if signs of ill health are observed, animals will be culled immediately. During the previous licence, no animal had to be killed due to the presence of ulcerated tumours. All animals will be humanely euthanised at the end of the experiment, or earlier if the humane endpoints are reached first.

Replacement

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

The experiments using animals will tightly interconnect with experiments performed using cells (*in vitro*). To partially replace the use of animals, we have found a way to mimic the early phases of cancer development by growing cells in a plate. Most of the work investigating CSC formation will be done using this system. However, to fully understand how CSC function within established tumours and how they drive disease relapse we will necessarily have to perform experiments in mice (*in vivo*), since we need to analyse tumours in their natural context. Nevertheless, most of the experimental measurements and the analysis of tumours will be performed after tumour removal post mortem.

Reduction

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

Our use of *in-vitro* approaches limit the numbers of animals required for the *in-vivo* investigation stage. In addition, when dissociated tumour cells are not in use, they will be stored in a frozen state. This minimises the numbers of animals required for maintaining live tumour cells. When animals are needed, we employ several strategies to try to limit the number of mice in the study. Firstly, we always aim to maximise the amount of data we get from each mouse, for example by injecting cancer cells in multiple sites to induce two tumours/mouse. Also, we will limit the use of genetic models (that often require many generations breeding) using transplants of cells and treating the mice with chemical agents to generate tumours. We also use the minimal amount of mice needed for statistical significance when testing the experimental hypothesis. Furthermore, we will use in vivo imaging, which allows us to use the same animal for repeated measurements and reduces the overall number of animals needed. Finally, by careful monitoring of our mouse colonies we try to breed as few mice as possible.

We will also collaborate with other groups, sharing data and animal tissues, in order to minimize the overall number of animal used.

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.

For our studies we will use mice, because tumours grown in mice are very similar to human tumours and discoveries made using mouse cancer models can thus inform us on the human disease. Furthermore, many experimental model are already available and refined techniques have been developed. The first approach used in the project will mainly use mice lacking a functional immune system, which allow tumour formation by injection of human cells, or standard laboratory mice that will be injected with mouse cells. Animals will be housed in highly clean facilities to minimize the chance of infections. A second approach will use animals with genetic alterations that make them prone to cancer development or mouse that have been treated with chemical agents using refined and widely-used protocols. To minimize animal distress, in many cases we use genetic alterations that we can activate only when needed to perform the experiments. For all manipulations and procedures, we follow national guidelines that aim to minimise suffering. We have extensive experience with most procedures

described in the licence. When performing procedures in which we are not fully competent, we will seek the help of other groups in the institute that have optimized those procedures. To minimise any possible adverse effects of the experimental procedure, we closely monitor the animal's reaction to specific experimental procedures and pay attention to any sign of suffering. The use of specific treatments (when possible) or methods of humane killing will be used depending on need. Surgical procedures, when needed, will be done with suitable anaesthesia and animals monitored post-surgery to ensure that they recover well. We will also use suitable analgesia according to the procedure.