Home Office

NON-TECHNICAL SUMMARY

Investigation of pathways regulating tumour progression and regression

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, inflammation, oncogene, tumour microenvironment, tumour suppressor

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

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
- Required at inspector’s discretion

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 the cellular processes that contribute to cancer and identify components within these processes that are potential targets for therapeutic intervention.

A retrospective assessment of these aims will be due by 18 November 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's 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?

Despite dramatic improvements in the treatment of some cancers, many remain stubbornly refractory and even those that initially respond then frequently relapse. Relapse generally results through evolutionary selection of subclonal populations of tumour cells that carry mutations conferring drug resistance or by adaptive compensatory rewiring of functionally redundant intracellular pathways. Elucidation of the individual signalling pathways that govern the evolutionary trajectories of tumours from benign mutant pre-cancerous cells to a malignant, aggressive and heterogenous cell mass capable of rapid and continual evolution is paramount. The signalling programmes that instruct the development of solid tumours is closely related to an organ-specific physiological programme responsible for regenerating and repairing damaged tissues. The latter part of this wound healing process relies on a dedicated resolution programme that returns the tissue to its normal state - we have evidence that this same programme is engaged during tumour regression in response to targeted therapies. Elucidation of these programmes will provide invaluable insights for the development of novel and durable cancer therapies, for preventative strategies and in early detection.

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

Advances in fundamental knowledge

Cancer is a disease that arises through accumulating errors (mutations) in the genetic instructions that regulate and control how the cells in our tissues replicate, spread and die. In normal tissues the

processes of cell gain and cell loss are maintained in an exquisite balance: cells only replicate in the right place, at the right rate and at the right time. The process is controlled by genes that promote cell increase (oncogenes) and genes that prevent it (tumour suppressors) – these act as, respectively, accelerators and brakes. Mutations in many different oncogenes and tumour suppressors contribute to the development of different kinds of human cancers. Moreover, because these mutations occur and accumulate randomly, each cancer is different from every other and even within a cancer in a single patient there are many genetic clonal variants. It is this complexity and diversity of human cancers that confounds our ability to contain and treat the disease.

However, just because cancers harbour many differences from each other does not necessarily mean that they are functionally different. Although a particular make of car may have many drivers, all those drivers act through the same, common, engine. Our research explores the provocative idea that certain underlying essential processes (engines) are shared across many, perhaps all, cancers even though the mutations (drivers) that power those engines may vary from patient to patient. In this conceptual framework, the huge number and diversity of cancer driver mutations are a distraction. Hence, our aim is to identify the common cancer engines, determine what they do and how they work, and ascertain the therapeutic benefit of targeting them with drugs. Our ultimate goal is to accelerate the development of general anti-cancer treatment strategies that may be administered to patients irrespective of what type of cancer they have.

Our driving hypothesis is unorthodox and counters the prevailing dogma that cancers are irreducibly complicated. But it is backed by several decades of our research work, that has identified aberrations in the function of two pro-cancer oncogenes – a molecular switch called Ras and a molecular regulator of genes called Myc – and in one pivotal tumour suppressor – a stress and damage sensor called p53 – as common to many, perhaps all human cancers. However, none of these is yet targetable by drugs. Hence, our only means for deciphering the roles of these cancer engines is to use sophisticated, switchable mouse genetic models. These models allow us to reversibly switch Myc, Ras and p53 on and off in normal and neoplastic tissues and thereby directly determine what the roles of these engines are in the genesis and maintenance of different cancer types. Over the past few years, our principal focus has been on the Myc "engine," whose diverse activities appear to be absolutely fundamental to the genesis and maintenance of diverse cancers. We have painstakingly identified and mapped the web of interactions that link Myc activity to control of the tumour itself, as well as the pathogenic inflammatory and immune-suppressed tissue that surrounds and supports the tumour in its midst. Our mouse models also allow us to assess the therapeutic consequences of manipulating such common cancer engines: would interfering with their function be therapeutically effective? If so, why? And how toxic might the side effects of such interference be for a cancer patient. In this way, we will establish in principle what are the most effective and tumour-specific cancer therapeutic targets, so informing pharmaceutical strategies for future cancer therapies. We will also test existing therapies in these same controlled and reproducible disease models. The data generated will be used by chemical engineers to guide the design of completely new drugs of general applicability across a wide range of neoplastic diseases.

Production of valuable resources

We have developed and validated some of the core regulatable genetically altered mice that we will utilize in our future studies. Additional novel mouse strains will be developed and used in conjunction with existing strains and with those available to us from commercial sources or from collaborators.

Given our proven track record in the development of novel switchable genetics in mice, we are highly likely to generate significant results. Our results will be published in influential peer-reviewed journals and disseminated through scientific seminars. Novel regulatable genetically altered animals developed during the course of this project will be made freely available to other researchers interested in the functions of the target proteins in normal adult tissue and in cancer and other diseases.

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

It has always been our strict policy and practice to freely distribute all data and reagents, including genetically modified mouse strains, without conditions and prior to publication. This policy will also apply to scientific outputs covered by this project. We are investigating the possibility of depositing our mouse strains with the European Mouse Mutant Archive (EMMA/Infrafrontier). There will be no moratorium on presenting our research at national and international scientific meetings and via bioRxiv (https://www.biorxiv.org) prior to submission to open access peer-reviewed journals.

Sequence data will be maintained by the host institution and, in addition, RNAseq and ChIPseq data will be submitted to publicly accessible databases such as ArrayExpress (http://www.ebi.ac.uk/arrayexpress/). Other data such as mouse strain, husbandry and genotyping are stored on local databases but relevant information will be made available in suitable formats on request.

Where data are not available through public databases, interested parties will be provided with a secure digital links to the requested data via the host institution.

In the longer term, our hope is to stimulate increased academic, biotech and pharma efforts towards inhibiting the Myc oncogene and identify Myc effectors, crucial for initiation and maintenance of tumours, that are potential therapeutic targets.

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

All our research will be routinely presented at national and international scientific meetings and via bioRxiv (https://www.biorxiv.org) prior to submission to open access peer-reviewed journals. Our group participates in many collaborative endeavours, some of which use mouse models developed under our existing PPL. In addition, the applicant and other lab members are frequently invited to present at scientific meetings throughout the year at which expertise on the animal models is freely communicated.

Species and numbers of animals expected to be used

- Mice: 41000

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.

The aim of this project is to elucidate the contribution of the Myc protein to tumourigenesis, tumour maintenance and normal physiology and to validate Myc as a potential therapeutic target for cancer. Since cells cultured in vitro do not adequately recapitulate the cellular context and interactions in which cancers evolve it is necessary to employ animal models. For example, interactions between the tumour cells and several different types of non-tumour (stromal) cells that constitute the "tumour microenvironment" are crucial for tumour growth and survival. Inhibition of the soluble signals that drive these interactions (and those that instruct tumour regression) offer a promising basis for tumour therapy that can only be elucidated in the whole organism, particularly since some of the stromal cell types recruited to the developing tumour originate in distant organs. All of the proposed animal experiments involve mice. The mouse is the most suitable model system in which to perform these studies. Our major interest is in adult human tumours (particularly those arising in the lung, pancreas and liver) and our experimental mouse models generate tumours in these organs of adult mice that closely resemble the human disease. Moreover, the mouse genome is exceptionally well characterized and can be manipulated by gene targeting and there is extensive knowledge on the breeding and husbandry of rodents. The animals will be maintained in Home Office-approved facilities offering rigorous guidelines to ensure the best welfare and so that animal numbers are kept to a minimum.

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

Typically, non-toxic gene/protein activating agents will be administered to genetically altered mice, either in drinking water or in their diet. In a minority of cases such agents will be administered by gavage or injection (usually intraperitoneal). Administration of such substances induces tumour development. In some cases, a potential therapeutic compound is administered at the same time to determine its effect on tumour development. In addition, a small number of mice spontaneously develop tumours after several weeks/months. Our experiments last a few days/weeks and we always aim to limit the tumour size to minimise suffering prior to switching off the activity of the gene/protein and/or administering a therapeutic agent to assess tumour regression. One important hypothesis, for which we already have convincing preliminary evidence, is that tissue-specific programmes that instruct tissue regeneration and resolution in response to injury are hijacked by oncogenic proteins. Thus, some animals will be subject to tissue-specific injury to understand these processes and how oncogenic proteins are involved. For example, liver is the principal organ involved in de-toxification of hazardous compounds and, as a consequence, has evolved a remarkable capacity to regenerate. Continuous chronic assault on liver from hepatotoxic agents such as alcohol, mycotoxins or natural alkaloids drives a sustained cycle of damage and repair that is thought to contribute both to liver failure and liver cancer. We will use controlled, sub-lethal doses of CCl4 to elicit acute liver injury and then monitor the roles of Myc and Ras oncogenic signalling pathways in instructing the rapid repair and regeneration of the organ. Pancreas is another tissue of interest where overlapping molecular programmes appear to underpin both repair of pancreatic injury and pancreatic adenocarcinoma, a cancer with dismal prognosis. Acute or chronic injury in pancreas will be induced by intraperitoneal injections of cerulein, a cholecystokinin (CCK) analogue that enhances secretion of digestive enzymes from the pancreas acinar cell and acutely induces mild to moderate acute interstitial pancreatitis. Recovery from each cerulein dose is rapid and involves regeneration and remodelling of pancreatic exocrine tissue that transiently shares great mechanistic overlap with pancreatic adenocarcinoma. Bleomycin, derived from Streptomyces verticillis, is a potent peptide toxin by virtue of its inherent pyrimidine and imidazole

structures, which act as iron-dependent oxidants. Bleomycin has particular toxic activity against lung type II alveolar cells (the cell of origin of non-small cell lung adenocarcinoma) due to the tissue's relative deficiency in the deactivating enzyme bleomycin hydrolase (Hay J, Shahzeidi S, Laurent G. Mechanisms of bleomycin-induced lung damage. Arch Toxicol. 1991;65(2):81-94. doi: 10.1007/BF02034932. PMID: 1711838). In all these instances, injury is transient, rapidly repaired, and most mice recover within a few days. At the end of the experiment the mice are humanely killed and molecular analyses conducted on multiple tissues.

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

Our experimental models are designed to induce tumours in lung, pancreas, skin, breast and B cells or tissue damage in lung, pancreas and liver of mice that will cause the least suffering for the shortest period. Nonetheless, the nature of the experiments (tumourigenesis and tissue injury) will cause inevitable adverse effects and mice may suffer transient weight loss, tumour development (which in the lung may lead to respiratory distress) and pain (largely confined to pancreatic tissue injury).

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)?

Our aim is to minimize animal suffering commensurate with directed scientific experimental design and statistically valid output. Indeed, we would argue that animals experiencing persistent pain are not a good experimental model. Nonetheless, our experimental models are designed to induce either tumours in lung, pancreas, skin, breast and B cells or tissue damage in lung, pancreas or liver of mice, and this will likely cause at least transient suffering. Since the majority of our genetically altered animals harbour switchable alleles, we can accurately and reproducibly switch on (and off) key proteins that govern tissue regeneration/tumour progression and wound resolution/tumour regression, allowing us unprecedented accuracy in predicting the kinetics of tumour growth (and regression). This allows us to either kill the animal before severe adverse effects are manifest or initiate tumour regression and thereby forestall any adverse impact. The majority of animals, such as the 60% used for breeding, will not exceed mild severity. Experimental animals will experience moderate severity (20-30%) and some animals (<2%) may experience severe adverse effects. For experiments designed to induce tissue damage in a single organ (lung, pancreas, liver) we aim, via reductions in dose, to induce the minimum damage that initiates a regenerative response. The damage inflicted is acute, tightly controlled and transient and the mice recover completely within a few days. Lung damage: we intend to administer bleomycin by intratracheal instillation or inhalation (Izbicki, G et al 2002. The course of bleomycin-induced lung fibrosis. Int. J. Exp. Path 83, 111-119) and will seek additional advice and expertise on this method. Pancreas damage: cerulein is a cholecystokinin (CCK) analogue that enhances secretion of digestive enzymes from the pancreas that induce a form of self-injury. Cerulein-induced damage to the pancreas may be acute or chronic and, while widely used to cause pancreatic damage and induce pancreas inflammation and injury (akin to the human condition pancreatitis), the effects on the tissue and adverse effects to the animals can be variable and a small percentage (~4%) of animals may experience severe effects for short periods (before being killed). Toxin-dependent liver damage causes short-lived moderate adverse effects and the liver very rapidly recovers both function and form.

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

- Used in other projects
- Killed

A retrospective assessment of these predicted harms will be due by 18 November 2025

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?

The aim of this project is to elucidate the contribution of Myc to tumourigenesis, tumour maintenance and normal physiology and to validate Myc as a potential therapeutic target for cancer. Since in vitro studies cannot adequately recapitulate the physiological and systemic context and interactions in which cancers evolve it is necessary to employ animal models. For example, interactions between the tumour cells and several different types of stromal cells that constitute the "tumour microenvironment" are crucial determinants in tumour evolution, growth and survival. Inhibition of the soluble paracrine signals that inhibit these interactions is a promising basis for tumour therapy whose impact and tumour dependence can only be investigated in the whole organism, particularly since some of the key stromal cell types (i.e. lymphoid and inflammatory cells) are recruited to the developing tumour from distant organs.

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

Where possible, our animal studies are replaced and/or complemented by cell/tissue/organoid culture experiments using both commercially available and mouse-derived established cell isolates. These studies are invaluable in investigating the cell autonomous nature of cell signalling and cell processes but cannot address complex interactions between multiple cell types. We will consider the use of 3D organoid models that have been developed for lung, pancreas and liver (three prominent tissues in our studies) but these still fail to address the inherent difficulties of modelling complex and highly tissue-specific interactions between tumour and normal cells that we know is critical to cancer growth and response to therapy *in vivo*.

Why were they not suitable?

Cells in culture are subject to an environment very different from that *in vivo*. They experience a variable and abnormal oxygen tension that is only poorly controlled and defined, are usually cultured in

a vast excess of glucose and ill-defined mitogenic growth factors, survival factors, cytokines and chemokines of (typically) bovine origin. Their rapid proliferation in culture is abnormal and facilitates rapid evolutionary selection (e.g. for cells that proliferate faster and/or are more resistant to cell death). Although organoid cultures offer some improvements, they are severely limited by the rather crude and unrepresentative matrices (e.g. Matrigel) that must be used and which do not reconstruct the complex interaction of multiple cell types. For example, a functional dynamic immune and inflammatory system that responds to, and is instructed by, the tumour cells - a central mechanistic tenet of our research - cannot be reconstituted *in vitro*.

A retrospective assessment of replacement will be due by 18 November 2025

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?

The aim of this project is to elucidate the contribution of Myc to tumourigenesis, tumour maintenance and normal physiology and to validate Myc as a potential therapeutic target for cancer. We will use well-validated mouse models with which we have extensive experience. There is considerable overlap in our experimental design that serves to reduce total numbers of animals used.

Mice will be randomly allocated to experimental groups, maintaining a comparable segregation of age, size and gender. All animals will be maintained in the same environment. Details of the random allocation will be retained by professional biological resources personnel or our existing chief animal technician not directly involved in this project. The same person(s) will administer experimental agents (or control substances) and retain a key to identify recipient mice. This key will only be accessed after analysis has been completed.

For most experiments we calculate that 5 - 6 animals are required per group to generate biologically meaningful and statistically valid data. This is based on a range of considerations agreed with our advisory biostatistician.

Since most of our studies are based on the dynamic changes that follow Myc activation, we will generally require 3 time points per experiment (typically, but not exclusively 1, 3 and 7 days) making a total of 30 animals per experiment. In this case the choice of the test of interest might be to compare with and without treatment, at different time points, and across time points (all treated). Again, all known

statistical considerations will be implemented to be sure to derive the maximum usable data from each experiment.

Since many of our experiments require animals with complex genetic makeups, we will carefully plan breeding strategies to minimize the number of animals of incorrect genotype generated. For example, some experiments require animals with a specific combination of 5 or more alleles. Hence, more than 65% of the animals will be involved in breeding protocols only.

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

Calculations are informed by reference to Festing, MF and Altman, DG, 2002 (ILAR J 43, 244-258) and NC3Rs Experimental Design Assistant (https://eda.nc3rs.org.uk/eda/landing) and with assistance from in-house biostatisticians dedicated to optimising use of animals in experiments.

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

Since most of our experimental animals have complex genotypes, we have carefully planned breeding strategies to maximize the number of suitable experimental animals and control littermates. All animals will be humanely destroyed at the end of experiments and tissue samples taken for further experiments. Where possible (e.g. where tissues can be used as controls for other experiments), mouse tissues will be shared amongst the research group. This will maximize the amount of information that can be acquired from the minimum number of animals. Where appropriate, pilot studies will be conducted to determine feasibility and efficacy.

A retrospective assessment of reduction will be due by 18 November 2025

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.

One of the problems with classical transgenic and xenograft mouse cancer models is the unpredictable and highly variable time it takes for tumours to emerge. In such instances, it is unknown why some

animals take longer to exhibit disease than others. For this reason, all animals have to be constantly monitored and their state of health inferred indirectly by external signs. Such unpredictable variability makes minimisation and mitigation of adverse effects much more difficult. The great variability in the time to tumour presentation in such classical mouse models also means that the critical events that eventually drive overt outgrowth of tumours, and timing and sequence of such events, are very poorly defined, making it almost impossible to establish the cause-and-effect events that cause the cancer.

By contrast, our sophisticated switchable genetic technologies allow us to regulate expression or activity of key cancer genes at will, in real time in target mouse tissues. This triggers tumour formation and regression with highly reproducible, consistent, predictable and rapid kinetics. This allows detailed assignment of which, when and how the various oncogenic events accumulate to cause cancers while greatly reducing numbers of animals needed in each cohort to achieve statistically valid data. Moreover, because of the highly predictable latency and rate at which our models develop tumours, we are able to terminate most experiments at a very early, incipient, stage when tumours are small and have not yet spread or metastasised. Consequently, most mice exhibit only moderate adverse effects and the small number that exhibit severe effects will be killed immediately.

Our studies on the role played by the Myc protein in tissue regeneration, and its relationship to cancer, requires animals in which different single tissues are deliberately damaged. Although not causing lasting harm (the tissues rapidly and efficiently regenerate), these models may generate transient suffering and pain. Where possible the pain is mitigated by analgesics.

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

Our scientific goals rely on being able to model the development (and regression) of adult human cancers in the mouse. Thus, it is not possible to use more immature stages or terminally anaesthetised animals. Mouse physiology is sufficiently similar to that of humans to generate passable representations of the human disease. This is facilitated by detailed knowledge and comparison of mouse physiology and genetics. Tumourigenesis is a dynamic process, taking several years in humans - in the mouse, we can speed up and precisely regulate this process using a number of genetic manoeuvres such as reversibly switchable protein activity and/or gene expression.

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

We will continue to use rapidly switchable models that allow short-term, consistent and predictable outcomes that allow us to limit welfare costs to the animals.

For example, in some experiments we employ the commonly used pdx1-Cre allele to activate expression of oncogenic Ras and Myc proteins specifically in the pancreas. However, we have observed extra-pancreatic expression in this mouse model leading to collateral adverse effects, specifically hyperplasia and neoplasia in the intestine. For this reason, we developed a replacement mouse model in which expression of Ras and Myc is tightly restricted to the pancreas and extra-pancreatic adverse effects do not occur.

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

We adhere to the guidelines published in Workman *et al*. (2010) Guidelines for the welfare and use of animals in cancer research. BJC 102, 1555 - 1577 and the NC3Rs ARRIVE guidelines.

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

We are advised of advances in the 3Rs via regular correspondence (email). Furthermore, our dedicated technician attends relevant 3Rs - sponsored meetings. Information is disseminated to the rest of the group. In addition, one of our colleagues has a research project funded by the NC3Rs to develop organoid models and refine existing models of lung cancer.

A retrospective assessment of refinement will be due by 18 November 2025

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?