Home Office

NON-TECHNICAL SUMMARY

Therapeutic implications for inflammation-driven cancers

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
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

cancer, therapy, liver inflammation, liver injury, metabolism

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, 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 outline the impact of anti-apoptotic genes in cancer development using models of inflammation-driven cancers (e.g. liver inflammation and cancer).

To determine cellular and molecular mechanisms which regulate inflammation, tissue regeneration in response to injury and cancer development

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?

Around half of cancer patients in the UK now survive their cancer for more than ten years. But whilst outcomes for some types of cancer have improved enormously, patients with other tumour types continue to do very poorly. And once cancer has spread around the body, it is still often incurable. An example is given by hepatocellular carcinoma (HCC), a complex tumour of the liver that is most commonly associated with underlying chronic liver inflammation (risk factor).

Genomic analyses have provided a clear picture of the main molecules driving liver tumour initiation and progression. However, only a handful of these drivers are being used as pharmacological targets, which limit their applicability in the clinic. Therefore, there is an urgent need to find additional targets in the pathways that regulate the development of this deadly disease.

Hence, the new insights into how liver cancer develops are expected to have a significant impact on either the prevention (chronic inflammation) or targeted inhibition of the advanced disease. Our goal is to exploit the findings made in animal models of inflammation-driven HCC for rapid translation into the clinic.

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

Our proposed studies are aimed at a better understanding of the causes and biology of the development of HCC, an incurable tumour of the liver classified as a cancer of unmet need.

Cancer and chronic inflammation represent a considerable health burden in society. A major goal of the proposed program of work is to test the role of key signalling pathways that are implicated in the multistep process of carcinogenesis induced by chronic inflammation.

For example, using GA mice harbouring deletion of anti-apoptotic genes, we can test whether modification of specific signalling proteins alters the ability of generating chemically-induced or spontaneous tumours, in vivo. By studying the impact of perturbing cell signalling pathways regulating apoptosis and cell survival, we can gain insights into the molecular and cellular mechanisms underlying the development of chronic inflammation and cancer. This knowledge might help inform our design of drugs to improve immunity to pathogens (resolution of inflammation) or lead to new insights for potential therapeutic approaches for the treatment of cancer. Given the complexity of the interactions of the inflammatory cells with the tumour microenvironment, in vivo models to study to the impact of signalling pathways are required. However, these experiments will be complimented by detailed analysis of cancer cells responses in in- vitro cultures, as well as biochemical, molecular biology and proteomic approaches.

Important and immediate outputs for the work will include advancement of scientific knowledge in inflammatory disorders and associated development of cancer. The project will help identify novel approaches to manipulate the inflammatory responses. Thus, it is expected that the knowledge gained from our animal experiments will be translated to pre-clinical human studies. A specific benefit would be to understand how inflammation drives the development of tumours with the aim to inform drug companies of the physiological importance of certain anti-apoptotic genes of interest to generate selective drugs to stop tumour development.

In addition, the transgenic mice development carried out in this project licence will be valuable to other scientists interested in developing anti-inflammatory or anti-cancer therapies.

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

In the short term, the results from our proposed project are directly relevant for academic researchers working in the fields of cancer signalling and drug discovery. The results originating from our research may spur collaborations with pharmaceutical companies which have efficient pipelines to screen for and test drugs or biologicals against putative targets, or available drugs could be repurposed for liver chronic disease and/or HCC therapy.

Our ultimate goal is to improve the health and wellbeing of patients by preventing the formation of liver cancer (via modulating liver injury and inflammation, two major risk factors of HCC development) or by suppressing advanced stages of HCC.

In the long run, it is anticipated that patients would benefit from the insights resulting from our proposed research, which will have sparked translational work by us and other national and international academics through the publication of the results.

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

External dissemination of our results to academics and clinical scientists will be achieved via peer-reviewed publications and presentations at national and international scientific meetings, including hepatology and cancer meetings.

There is a real prospect of translating our findings on novel potential targets for pharmacological inhibition into clinical trials, since several investigators and liver surgeons (collaborating with our team

and involved in running national clinical trials for HCC) are interested in the development of improved treatments.

For non-academic users, we propose to communicate our research through a series of Health New outlets as previously realised by the lead investigator and co-investigators. On this regard, the applicant is an active science-communicator at "The Conversation UK", a global media resource providing informed commentaries and debates on the issues affecting our world. He has published several articles in the “Health” section that have been read and shared on social media.

Species and numbers of animals expected to be used

- Mice: 4000

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.

In the proposed work we will use transgenic mouse models to investigate how different protective genes direct the development and progression of chronic inflammation to cancer. Some of these models are already available in our laboratory, while others are becoming available elsewhere, involve the interplay of multiple tissues and factors that cannot be modelled using currently available tissue culture systems.

The availability of resources for genetic manipulation and phenotyping underlies the choice of the laboratory mouse for this work. Over decades of research, this approach has generated a wealth of knowledge that has formed the basis for a great number of clinical applications in humans. No other species of lesser sentience would fulfil the criteria for this programme of work to the same extent.

Of exceptional importance for the planned studies is the fact that the immune system of mice is well characterized and closely resembles that of the human, which has the important implication that insights from mouse models can be directly translated to humans. Indeed, the mouse and the human genome are the most highly homologous genomes of the large vertebrates.

Specifically, the transgenic mouse models described in this project are required to test whether specific signalling pathways and molecules play a biological and functional role in the in-vivo response to liver injury, regeneration and development of cancer.

In our models we will employ both early young mice (14-days post-birth) and adult (8 weeks-old to 10 months-old) mice given that a precondition for the planned studies is to understand the stages of cancer development from the start of the life throughout the entire of adult life, which mimics the actual timeline of humans being exposed to different lifestyle choice.

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

The proposed study will employ genetically modified animals (GMAs) and therefore tissue biopsies will be taken by ear punch and subjected to molecular genotypic analysis to determine the genetic status.

Some mice will be administered substances, such as hepatotoxin (i.e., CCl4 or TAA) by intraperitoneal (most cases) or intravenous (rare cases) injection, in drinking water or via diet supplementation. Some mice will receive a single (acute, high) dose of hepatotoxin to test the acute response to liver injury within 48-72 hours post-administration. Some other mice will receive a repetitive (usually once a week for up to 6 weeks) (chronic, low) dose of hepatotoxin to test the regenerative capacity of the liver.

A typical experiment consists of injecting carcinogens to 14-days old mice and when adult they will be freely put on either standard diet or a modified diet with high-content of fat, to check how the combination of carcinogens (first hit) and the different lifestyle choice (second hit) would impact the development of cancer in certain GM animals. Following this regimen for up to 10 months of age, animals will gradually develop tumours (tumours at this stage are clinically silent and therefore only a low percentage of animals are expected to show clinical signs of disease)

Small amounts of blood may be taken from a superficial vessel (e.g. tail vein) throughout the experiment. The purpose of the blood withdrawal is to determine the blood sugar content in some experimental mice.

Administration of adeno-associated virus-mediated expression of cDNA may be also used to deliver ectopic cDNA directly in certain type of cells (i.e. liver cells) without apparent damage to the target tissue

In certain experiments we may also employ the administration of substances such agents that label proliferating cells (e.g. BrdU, EdU) or drugs (chemical compounds) that rescue (alleviate) the symptoms of liver injury induced by liver toxins and/or diet.

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

Breeding and generation of genetically modified animals (GMAs) that will be used are generally vital and present no spontaneous development of disease. Therefore, GM animals bred and produced are not expected to exhibit any harmful phenotype.

Animals with altered immune status will be maintained in a barrier environment thereby minimising the likelihood of compromising health.

After injection of carcinogens some animals have the potential to develop a harmful phenotype, eg tumours, neurological signs, after a certain age (over 12 months). However most of the experiments will be terminated up to 10 months of age and therefore administration of these substances will have minimal impact on their wellbeing.

Administration of hepatotoxin (i.e., CCl4 or TAA) causes hepatic necrosis. From previous experience we know that the vast majority of animals tolerate single acute dose for up to 72 hours or multiple (chronic) intraperitoneal injections for up to 6 weeks of CCl4 or TAA. There is the possible development of pyrexia and weakness without local pain (<1%). During chronic studies, the animal may develop jaundice (more obvious in albino animals) and ascites. However, the liver should not fail at the doses

used and at no time will signs of hepatic encephalopathy occur (such as incoordination, circling movements and being comatose).

The adverse effects of dietary interventions could be the development of diabetes, in which liver may be involved. Longitudinal measurement of body weight and blood glucose test can pick up changes at an early stage thus implementation of humane endpoints can be done accurately.

Administration of tissue labelling substances such as BrdU, do not lead to any unfavourable adverse effects.

Administration of adeno-associated virus-mediated expression of cDNA is used to deliver ectopic cDNA directly in certain type of cells (i.e. liver cells) without apparent damage to the target tissue. We have extensive experience in this technology, and have not observed significant adverse effects of adenoviruses in livers.

All intra-peritoneal injections will be carried out by experienced personal licence holders and animal will be monitored for any signs of pain post-injection.

In all cases general clinical signs (such as Inactivity, Isolation from cage mates, Pinched face, narrowing of the eyelids, Discharge eyes/nose, reduced grooming, Scratching, Abnormal breathing, reduced food or water intake, Hunched posture, boarding of abdomen; aggressiveness, abnormal Body weight changes) will be used to indicate the wellbeing of the animals. Any animal that displays one or more of the above clinical signs will be immediately killed by a Schedule 1 method if its condition threatens to exceed the moderate severity limits in place in the experimental protocols of this licence.

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)?

For mice breeding and maintenance it is expected to have a mild severity (20% of cases) and sub-threshold (80% of cases).

All the experimental protocols fall under the category moderate severity (30% of cases) since a fraction of mice may develop inflammation followed by induction of liver fibrosis and generation of tumours (either induced by carcinogens or following an injury). The remaining 70% would experience a mild severity.

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

- Used in other projects
- Killed

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?

Our aim is to understand complex biological processes in primary cells, as this information will be most relevant to the design of future therapeutic approaches. Whilst some basic questions can be addressed using immortalized cell lines, such approaches have numerous limitations, most importantly that these cells are by definition quite distinct from primary cells. Immortalized cell lines frequently have oncogenic mutations in key signalling proteins, such as tyrosine kinases or small GTPases, which enable their survival and growth in vitro. As such, these alterations in key cell signalling proteins render the analysis of signalling transduction in cell lines as quite distinct to primary cells.

Wherever possible, we employ ex vivo techniques to study immune cell function; e.g. co-culture of lymphocyte populations with tumour cells in vitro. However, to understand the complex interplay between immune cells, cancer or toxic agents requires the use of in vivo models. Indeed, the complex cell-cell interactions that govern the initiation, amplification and resolution of an inflammatory response cannot be studied outside of an animal and only jawed vertebrates have B and T lymphocytes, which play a central role in regulating this response.

Therefore, for much of the proposed work we will use mouse models to investigate how different protective genes direct the development and progression of chronic inflammation, immunity and cancer. These diseases involve the interplay of multiple tissues and factors that cannot be modelled using currently available tissue culture systems.

With this in mind and to best of our knowledge, there are currently no non-sentient alternatives to the use of animals for our work.

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

For some aspects of our study regarding the gene/pathway validation studies as well as e.g. the investigation of the deregulated function of proto-oncogenes, whenever possible we plan to investigate those at the molecular level using appropriate in vitro cultures of human cell lines.

In complementary studies we are also employing the use of hepatic organoids, functional three-dimensional (3D) in vitro models of the liver that may serve as a novel platform to address diverse research questions pertinent to hepatic development and regeneration, detoxification and metabolism studies, liver disease modelling, and adult stem cell biology.

Why were they not suitable?

Until now, no functional in vitro cell culture system has been developed that faithfully mimics all aspects of the in vivo liver reaction. A major caveat is that the in vitro cultured cells do not mimic the microenvironment of the liver cells within its tissue. Thus, in the absence of an in vitro system for the highly complex process of liver cancer development, the use of animal models remains the only rational approach to study the distinct stages of chronic inflammation and cancer development in the context of the complex living organism.

Liver organoids have major limitations that include: a lack of organotypic features, as compared to in vivo (i.e., the lack of inflammatory cells, the lack of the perisinusoidal space (or space of Disse) where hepatic stem cells would reside, along with a non-physiological ratio of hepatic stem cells to hepatocytes (HSC making up 50% of the spheroids, compared to 5–8% observed in the liver)

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?

Our previous experience allows us to ensure that animal usage is kept to a minimum and experiments designed in such a way that we expect the outputs to be measurable and reproducible. Established breeding colonies are, where required, maintained as homozygous lines such that all animals produced are of the desired genotypes.

The proposed experiments are based on the decade-long experience in the administration of hepatoxins to induce these models. We therefore do not anticipate carrying out pilot studies. However, should less invasive substances for the induction of fibrosis or its modulation become available during the project, we will determine the suitability of those substances by conducting small scale pilot studies and comparing results with our established protocol.

In order to achieve statistical significance and experimental reproducibility we typically perform an establishment (pilot) study by using small number of mice (typically 5-10 mice per group). This will help us to keep the animal numbers at minimum while testing the value of our experimental protocol.

Subsequently, we perform an efficacy study and the total number of animals is determined by G-power calculation, based on similar studies performed in other GA models. The numbers of mice to be maintained and used in experiments will be kept at an optimal minimum to ensure that reliable experimental data is obtained.

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

For all experiments, a randomized block design approach will be used and scoring will be “blinded” to the user. We will choose animals of similar weight and age. All the experiments will be performed in a ‘clean’ and a non-stressful environment reducing the risks of clinical or sub-clinical infection. All our animals are of the same genetic background, therefore, we will control the genetic variation using inbred strains.

In all experiments we will refer to the Experimental Design Assistant (EDA) available on the NC3Rs website.

Moreover, we follow the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines to ascertain that our research design is compatible with the ARRIVE checklist of recommendations.

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

Our project includes efficient breeding to reduce the number of GMAs that are not of the desired genotype, which is a general caveat of the interbreeding of multiple alleles. For example, in the experiments with the experimental cohort of geneXfl/flgeneY-Cre (tissue specific knockout), geneX$^{fl/+}$geneY-Cre and geneX$^{+/+}$geneY-Cre, we follow a breeding strategy in which geneX$^{fl/fl}$ mice are intercrossed with geneX$^{fl/+}$geneY-Cre mice to obtain Cre-expressing homozygous and heterozygous mice for the floxed allele, and geneX$^{+/+}$geneY-Cre with geneX$^{+/+}$ mice to obtain the Cre-expressing control mice. This strategy, which is possible to follow since all mouse lines are on the C57BL/6 genetic background, considerably reduces the generation of ‘unwanted’ genotypes that would have to be humanely culled.

Use of non- invasive in vivo imaging can also pick up progression of pathological changes in longitudinal study in one animal. This allows consistency of humane endpoints, thus increasing experimental reliability and reduction of animal use.

Although the majority of our experiments are focusing on the liver response to injury, we will systematically collect most of the tissues in a single animal. This will allow us to have a tissue bank collection of animals following these protocols that can be shared with other groups and investigators.

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.

During the past 5 years we have generated the above GAA animal models and refined the experimental methodologies and found that 70% of all mice used on this project will fall under mild severity. The majority of animals produced under the breeding program will be used to supply tissues for in vitro analyses. The majority of mouse lines have no defects beyond alterations in or loss of immune cell populations and suffer no ill effects in an SPF animal facility.

New immune/inflammatory challenges might provoke unpredicted responses; pilot studies to assess possible adverse consequences are undertaken in these circumstances. For each protocol, specific details for minimizing animal suffering are given. In all cases, we will refer to the published literature for each model to minimize suffering to mice. For all procedures, we will apply the least invasive methods of dosing and sampling appropriate to the objectives of the experiment, including the use of

anaesthesia for humane restraint where necessary. For example, in animals with tumours induced by chemicals, tumour growth will be assessed by IVIS imaging of tumour cell bioluminescence. Sequential imaging of animals in longitudinal studies can pick up pathological changes at an early stage thus implementation of humane end-points can be implemented more accurately, and consistently resulting in refinement of experiments.

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

The availability of resources for genetic manipulation and phenotyping underlies the choice of the laboratory mouse for this work. Over decades of research, this approach has generated a wealth of knowledge that has formed the basis for a great number of clinical applications in humans. No other species of lesser sentient would fulfil the criteria for this programme of work to the same extent.

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

After administration of substances, animals are closely observed for adverse effects, and monitored daily for signs of ill health such as piloerection / hunched posture / lack of appetite / reduced activity. Animals that are subjected to procedures with potential adverse effects will be monitored more frequently as described within the protocol steps. This will allow prompt application of humane endpoints if required.

Animals on longer term studies will undergo an acclimation period of at least a week during which time they will be handled regularly.

Use of non- invasive in vivo imaging can also pick up progression of pathological changes in longitudinal study in one animal. This allows consistency of humane endpoints, thus increasing experimental reliability and reduction of animal use.

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

We follow the most recent Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines, which have been published:

- The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, **PLoS Biol 18:e3000410, 2020; PMID: 32663219; PMCID: PMC7360023**.

We also make use of the most recent updated guidelines on the welfare and use of animals in cancer research:

- Workman P, et al., Guidelines for the welfare and use of animals in cancer research. **Br J Cancer. 2010 May 25;102(11):1555-77.**

For guidance on administration of substances we follow the LASA GOOD PRACTICE GUIDELINES Administration of Substances (Rat, Mouse, Guinea Pig, Rabbit): https://researchanimaltraining.com/wp-content/uploads/2021/05/lasa_administration.pdf

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

We will make use of the NC3R website and available 3R online resources. Through our connection with other local and national research groups, we have the opportunity to discuss protocol-relevant issues that may benefit our protocols.