G. NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Linking immunity, inflammation, regeneration and cancer
Key Words	inflammation, cancer, apoptosis, tissue regeneration
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;

No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

All the cells in our bodies are programmed to die. As they get older, our cells accumulate toxic molecules that make them sick. In response, they eventually break down, clearing the way for new, healthy cells to grow. This "programmed cell death" is a natural and essential part of our wellbeing. Every day, billions of cells die like this in order for the whole organism to continue functioning as it is supposed to.

But as with any programme, errors can occur and injured cells that are supposed to die continue to grow and divide. These damaged cells can eventually lead to diseases, including cancers. For instance, in the liver, chronic unresolved inflammation is associated with persistent hepatic cell death and compensatory regeneration, leading to sequential development of fibrosis, cirrhosis, and eventually hepatocellular carcinoma (HCC), the main type of liver cancer. **A thorough understanding of the molecular basis of inflammation-associated neoplasia can lead to novel approaches to prevent and treat HCC.**

We have recently shown that a well-established anti-apoptotic protein is highly expressed in liver samples from patients with either cirrhosis or HCC. Importantly, cross-cancer analysis in human samples reveals that this anti-apoptotic protein is mutated and highly expressed in a wide-range of cancer types associated with chronic inflammation, including HCC. Altogether our published analyses *in-human* biopsies and *in-vitro* cultured cells point in the direction of this protein target being important as a biomarker of hepatic chronic inflammation and cancer. **However, whether the enhanced expression of this target protein in human tissue represents a correlative epiphenomenon or is causally linked to cancer pathogenesis is currently unknown.**

Animal models of hepatic inflammation and HCC offer a prototype model to study mechanistic and cellular aspects of inflammation predisposing to cancer, including cellular transformation, tumour initiation, promotion and progression *in vivo*.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

Our recent studies have mainly used human samples analyses and human-derived cancer cells and obtained clear data of the functional mechanisms of our protein of interest. However, the physiological role is missing. The proposal here relies on transgenic and knockout models to dissect the molecular basis of hepato-cellular carcinogenesis, liver regeneration, and the liver response to cytotoxic challenges, in vivo. We propose to use these models because they will give findings of physiological relevance, which is not the case for cell culture models, and will allow us to directly test hypotheses rather than obtaining correlative data. We feel that understanding how protein targets function at the molecular level in tumourigenesis will have an impact in several areas of cancer pathogenesis and may lead to the development of new therapies for treatment of human cancers.

What types and approximate numbers of animals do you expect to use and over what period of time?

Mice offer the advantage of the availability of an array of inbred and mutant and transgenic strains and molecular reagents, and avoid the use of more emotive vertebrate species. The number of animals planned for these studies is the minimum needed to achieve the goals of the project as calculated by power calculations. Whenever possible, number reduction will be further achieved by using the same animal for multiple analyses by dissecting the liver into fractions that are processed differently, according to the type of assay performed. In order to achieve statistical significance and experimental reproducibility we typically perform an establishment (pilot) study by using 5-10 mice per group. This will help us to keep the animal 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. In designing new experiments, we will perform power calculations and in some occasions, we may seek statistical advice on experimental design from Biostatistician. Example of power calculations for a single experiment: For carcinogenic studies power calculations will be performed as follow: assuming that a difference of 1.66-folds of tumor numbers between two groups (WT vs. KO) of male-only mice developing HCC is of biological importance, we have calculated a sample size of n=30 for each group of animals (mean-WT=10, mean-KO=6; s.d-WT=5, s.d-KO=3; effect size d=0.97; power of 0.95; two-tails; $P<0.05$).

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

Hepatotoxin administration in WT C57BL/6J mice promotes liver injury, fibrosis and ensuing development of tumours. For example, administration of DEN cause no major impairment of liver function and therefore is classified as a moderate procedure. At the endpoints mice will be humanly killed and explanted tissue (and cells) will be further analysed

Application of the 3Rs

Replacement

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

Replacement

The use of animals is an essential component to understand the cell types and molecules involved in the sequential process of inflammation-driven carcinogenesis. Indeed, there is no suitable *in-vitro* model to study these complex cell-cell interactions that govern the *in-vivo* processes investigated in this program.

If during the project new in-vitro models will be developed we could test it for replacement

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

In designing new experiments, we will perform power calculations and in some occasions, we may seek statistical advice on experimental design from Biostatistician.

For each experiment we will perform 1) pilot studies and 2) final optimized experiments.

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.

1. For pilot studies, a small number of animals will be used. According to our previous studies, six mice for each genotype will be injected with different hepatotoxins and development of disease will be detected at end-point. This will help us to keep the animal at minimum while testing the value of our experimental protocol.
2. 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.

In addition, for all experiments, a randomized block design approach will be used and scoring will be “blinded”. We will also refer to the Experimental Design Assistant (EDA) available on the NC3Rs website (www.nc3r.org.uk)

Refinement

Explain the choice of animals 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.

Refinement

Mice offer the advantage of the availability of an array of inbred and mutant and transgenic strains. No other species of lesser sentience would fulfil the criteria for this program of work to the same extent.

Explanted tissue will be used for different analyses and therefore one single mouse will give us different biochemical responses.

The severity limit for most mice will be mild (~80%). Thus, 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 suffers 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. For example, tumours induced by chemicals, tumour growth will be assessed by imaging analyses of tumour cell bioluminescence. In all cases, we will refer to the published literature for each tumour 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. 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.

Additionally, for some experiments only male will be used (see protocol 6) therefore to reduce the welfare and costs, female mice will be humanely killed after birth, if not needed for breeding purposes. This is because male mice develop about 50% more tumours than females. This finding is consistent with the higher rate of liver cancer in men than in women.