Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 16

Projects with a primary purpose of:
Translational and Applied Research - Human Cancer

Project Titles and keywords

1. **Gene transfer to rodents to test for adverse effects and correct disease**
    - Gene therapy, vectors, cancer, Friedreich ataxia.

2. **Evaluation of novel anticancer agents**
    - Evaluation, novel, anticancer, agents

3. **Regulatory mechanisms in normal haematopoiesis**
    - Stem cells, haematopoiesis, leukaemia

4. **Development of new biological anticancer agents**
    - Cancer, virotherapy, targeted, biologic

5. **Evaluating New Cancer Therapies**
    - Lung Cancer Models, Drug Discovery

6. **Thermosensitive nanoparticles for cancer therapy**
    - Cancer treatment, drug development, liposomes, theranostics

7. **Mouse models of pancreatic cancer and therapy**
    - Pancreatic Cancer, Treatment, Tumour stroma

8. **Anticancer Drug Discovery and Target Validation**
    - Anticancer, drug, discovery, target, validation

9. **Modelling bone-tumour interactions in metastasis**
    - metastasis, breast/prostate cancer, myeloma, bone

10. **Novel Biologicals for Tumour Immunotherapy**
    - Tumour Immunotherapy; Antibodies; Adjuvants; Targeted Delivery

11. **Signal transduction in lung cancer pathogenesis**
    - Lung, Cancer, Autophagy, NF-κB, Therapy

12. **Molecular imaging in cancer**
    - Molecular, imaging, cancer

13. **Development of new anticancer therapies**
    - Cancer, Drugs, Translation, Biomarkers

14. **Mouse models of tumour growth and progression**
    - Cancer, metastasis, therapy, transplantation

15. **In vivo imaging in cancer models**
    - Imaging, Cancer, diagnosis, therapy

16. **Cancer Therapy-Efficacy Licence**
    - Oncology, Tumour, Efficacy, Pharmacodynamic

17. **Understanding and targeting the drivers of malignancy**
    - Cancer, prevention, treatment, metastasis

18. **Regulation of Normal and Malignant Blood Cells**
    - Stem Cell, Leukaemia, microenvironment, niche, self- renewal

19. **Oncolytic HSV as an anti –cancer therapy**
    - Oncolytic , cancer therapy

20. **Stem cell function in tissue regeneration, diabetes and cancer**
    - Stem cells, cancer, diabetes

21. **Modelling cancer predisposition by BRCA2 mutations**
    - Cancer; Genetic alteration; Pancreatic cancer

22. **Genetic analysis of tumour development**
    - Cancer, tumour, inflammation, therapy, imaging

23. **Tumour models for therapy of advanced cancers**
    - tumour, metastasis, novel drugs, imaging

24. **Understanding inflammation, fibrosis and cancer**
    - Scarring, cancer, drugs

25. **Signalling pathways in cancer, inflammation, and metabolism**
    - NF-κB, cancer, therapy, inflammation, metabolism

26. **Platelets and cardioprotection in cancer**
    - Platelets, cancer, cardio-toxicity, metastasis

27. **Oncology models**
    - Oncology, Immunology, Therapeutics

28. **Haematopoietic and leukaemia stem cell regulation**
    - Haematopoiesis; Stem cells; Leukaemia; Lymphoma; Leukaemia stem cells; Novel Therapy

29. **Novel strategies to target cancer**
    - Cancer, Microenvironment, Immunotherapy

30. **Engineered mice for gene function analysis**
    - cancer, gene function, mouse, embryonic stem cells, embryonic development, developmental defect

31. **Studying the origins of cancers from stem cells**
    - Paediatric, adult, epithelial, cancer, biology

32. **Xenopus as a model for development and drug discovery**
    - Xenopus, developmental biology, Cancer, Stem cell biology

33. **Hypoxia and Angiogenesis in Cancer Therapy**
    - Hypoxia, Angiogenesis, Cancer

34. **Mechanisms of Metastasis**
    - Metastasis, myeloid cells, coagulation, extracellular matrix

35. **Induction of Anti-tumour immunity**
    - Cancer, white cells, immune-therapy

36. **Modelling cancer biology and therapy in mouse**
    - Breast mammary cancer metastasis xenograft

37. **Establishment of Patient Derived Xenografts from Biopsy Samples**
    - Cancer, tumour, biopsy, tissue generation

38. **Pancreatic Cancer: Biology and Therapy**
    - Pancreatic cancer, Pancreatitis, Therapeutic targets

39. **Colorectal cancer initiation and progression**
    - Colorectal, cancer, RNA, genes

40. **Model Systems to Improve Cancer Immunotherapy**
    - Cancer, immotherapy, mouse

41. **Implantable Microsystems for Cancer Therapy**

42. **Study and manipulation of immune-regulatory receptors to improve cancer therapeutics**

- Cancer, immunology, immunotherapy, checkpoint

43. Preclinical evaluation of cancer therapeutics

- Cancer, chemotherapy, immune-oncology

44. Personalised Tumour Graft

- Tumour, human, personalised

45. Assessment of novel cancer therapeutics

- Tolerability, Pharmacokinetics, Cancer, Mouse, Rat

46. Enhancement of targeted radiotherapy of cancer

- Targeted, radiotherapy

47. The molecular basis of lymphoma and leukaemia

- Lymphoma, leukaemia, mouse models

48. Pre-clinical assessment of new anticancer agents

- Oncolytic virus, immustimulation, vaccine, combination treatment, anti-cancer agent

49. Analysing cancer: immune cell interactions involved in metastasis

- Cancer, imagine, animal models, immune system, cell signalling

50. Generating New Mouse Models of Human Cancer

- Cancer, model, transgenic

51. Skin cancer survival in the aging population

- Melanoma, microenvironment, squamous cell carcinoma, aging

52. Mouse models for tumour stem cells and anti-tumour efficacy studies

- Cancer, brain tumours, tumour growth, invasion

53. Developing an ocular melanoma model for drug discovery

- Ocular melanoma, uveal melanoma, patient derived xenograft MEK

54. Adoptive T Cell Therapy for cancer

- Cancer, T lymphocytes, T-cell receptor, immunotherapy, adoptive therapy

55. Drug evaluation in pre-clinical oncology models

- Cancer, pre-clinical, efficacy, models, Imaging

56. Cellular Immunotherapy of Disease

- Gene-modified immune cells, tumour immunity

57. MYB Proteins and Myeloid Disease Susceptibility

- Stem cells, bone marrow, blood cells, leukaemia, ageing

58. Interrogating the Immunology of Cancer and the Development of Cancer Therapeutics

- Vaccine, therapeutics, cancer, immune cells

59. Pancreatic cancer – improving our understanding and therapeutic options

- Pancreatic cancer, therapeutic, genotype/phenotype

60. Vaccine Development and Immunotherapy

- **Cancer therapy, vaccine, therapeutic antibody**

61. Regulation of tumour growth and metastasis by sodium channels

- Antiepileptic drugs, breast cancer, invasion, metastasis, sodium channels

62. Immune cell mechanisms in cancer and infection

- Infection, Cancer, Immunology

63. Immune and Biological Therapies for Cancer

- Cancer, virus, immune system

64. Modelling a gene family in human disease

- Cancer, heart, skin, inflammation

65. Cancer Therapy – Enabling Licence

- Tolerability, Pharmacokinetics, Tumour, Surrogate

66. Sarcoma, bone niche, microenvironment and therapy

- Cancer, Microenvironment, Biomarkers, Therapy

67. Hematopoiesis in Development, Aging and Malignancy

- Stem Cells, Development, Leukemia, Aging, Transplantation

68. *In vivo* evaluation of enadenotucirev derived viruses

- Oncolytic, Adenovirus, Cancer, Immunotherapy

69. Normal and leukemic blood cell development

- Stem cells, Infant leukaemia, Transplantation

70. Regeneration/neoplasia of nervous system and muscle

- stem cells, organ regeneration, brain tumour

Project 1	**Gene transfer to rodents to test for adverse effects and correct disease**	
Key Words (max. 5 words)	Gene therapy, vectors, cancer, Friedreich ataxia.	
Expected duration of the project (yrs)	Five	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	Yes	Basic research
	Yes	Translational and applied research
	No	Regulatory use and routine production
	No	Protection of the natural environment in the interests of the health or welfare of humans or animals
	No	Preservation of species
	No	Higher education or training
	No	Forensic enquiries
	Yes	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aims of this project are threefold: I). To identify ways to improve gene therapy. II). To show gene therapy can be used for the correction of a neurodegenerative disease with the intention of showing this can be used in the future in the clinic and III). To understand what vectors cause liver cancer and the molecular events that are involved?	
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)?	From aims (I) and (II) we hope to show the carriers of genes (vectors) for therapy are effective enough to cure an inherited neurological disease for which mouse models are available, which is at present incurable. In aim III we wish to determine why some vectors have caused cancers in mice and humans in clinical trials and to use these vectors to identify new cancer genes. We hope the knowledge we will gain from this project will aid the identification of safer vectors for gene therapy and to provide information to other researchers for new treatments for liver cancer to be developed. Also, we wish to use this knowledge to design new *in vitro* assays to reduce and replace animals for this type of research in line with NC3Rs policy and to refine our procedures to achieve a reduction or replacement of animals for these important studies.	
What species and approximate numbers of	We will use only mice in this project, approximately 1,500 in	

animals do you expect to use over what period of time?	total over the 5 year period.
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 diseases we are studying can be fatal genetic diseases to children so we are using mouse models of these diseases. These mice can often fail to thrive, lose weight and become poorly. Some with neurological disease may start to show ataxia and possible heart failure. Mice treated with gene therapy vectors may develop tumours. However, we aim to be vigilant for such signs and have set up sensitive assays to detect these adverse effects early on and hence we will euthanize them if they do. The maximum expected level of severity is moderate. Mice will be killed at the end of the work and their tissues taken for analysis to determine correction of FRDA and the cause of tumour induction.
Application of the 3Rs	
1. **Replacement** State why you need to use animals and why you cannot use non-animal alternatives	Replacement plans for Objective 1 that aims to find ways to correct the inherited disease Friedreich ataxia using gene therapy or by introduction of disease correcting cells. Inherited genetic diseases involve many tissues and organs. For example, in Friedreich ataxia (FRDA) neuronal cell death is caused by lack of a gene called frataxin (FXN). However, the actual cause of patient death is by heart failure. In this project licence, the mouse models of FRDA have mild symptoms of the disease, which include reduced motor coordination and weaving gait. These mice can also show signs of heart wall thickening and can develop diabetes. Because of these characteristics, these mice will require continuous monitoring to prevent animal suffering. Any mouse that appears unwell so that we suspect that it may be suffering will be humanely put down at the earliest time possible. To investigate cure or treatment of FRDA in humans, we propose *FXN* gene or cell therapy. For this, we will need to show this is possible using the mouse models of FRDA. We propose to test for the best routes of injection of the FXN gene or cells that enables us to reach both the heart and neuronal cells or their compartments. Because some gene-carrying vehicles (therapy vectors) have been shown by our group to cause cancer we need to not only to show gene therapy works but also that it is safe and that cancer is avoided. It is important to us that we plan our experiments to replace the use of animals in our research where possible. To do this we always test our gene therapy vectors on cells in culture.

	This achieves the following: • It assesses vector purity, concentration and functionality i.e. its capability of infecting cells, hence reducing the wastage of animals in testing the vector and it also provides quality control to prevent animal sickness from impure vector batches. • We show the correction of FRDA at the molecular level in cells before testing this in the FRDA mouse models. Currently, we are also working towards using human stem cells (IPS cells) to mimic the correction of FRDA in the laboratory as a future replacement model. These experiments are aimed at obtaining information that mimics the animal experiments and/or complement animal experimentation and we always look for information on PubMed (http://www.ncbi.nlm.nih.gov/pubmed) on new procedures that can replace the use of animals for the above research. Replacement plans for Objective 2 that aims to understand what vectors cause liver tumours in mice and the mechanisms that are behind this. We have published findings that gene therapy vectors can cause tumours in mice when they are applied at an early age of development. Although we are developing tests using stem cells in culture to investigate what cancer genes become affected by these vectors, we still require studies to determine how tumours are caused in mice because this involves the immune system and tumours mainly occur in the liver. We have, from previous experience, knowledge of how to plan experiments in order to use the minimal number of mice for our investigations and we aim to use cells from these mice to replace the need for additional animals in this research. Hence, we hope to identify what causes cancer and replace the use of mice in our future studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	From previous experience, for most experiments, a pilot study using 4 animals per group is performed. In these studies we will use imaging to identify tumour development early on caused by gene therapy vectors. This will enable us to sacrifice animals before any suffering occurs. Using this protocol we are able to reduce the numbers of mice chosen for testing vector safety. This will also allow us to determine the spread of vector to tissues and organs as a result of the route of injection of vector used. Thus we reduce animal usage because we can determine this information without

	sacrificing mice. Data provided from pilot studies also enables retrospective power calculations to test whether sufficient animals have been used per group. The experiment may then be repeated in order to provide sufficient power. The type of statistical test to be applied usually dictates the experiment plan and, where appropriate, larger experiments utilizing the same control group are preferred to smaller experiments where a control group has to be included each time. From our previous work we have information on the number of animals and the length of time animals need to be kept alive to prospectively evaluate the numbers of mice we will use for experiments, using power calculations. We are fortunate to have this experience and statisticians at this University to aid our statistical analysis.
3. 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 the research to find ways to correct the inherited disease Friedreich ataxia using gene therapy or by introduction of disease correcting cells, we have chosen the FRDA mouse models as they have a mild FRDA disease phenotype with only reduced rather than lost motor coordination for which we have expertise in monitoring their health. Our collaborations will provide us with training on the handling of these mice and their monitoring to prevent animal suffering. We are, therefore, confident that we will be able to identify the signs associated with this disease early in order to perform humane killing so that we can harvest mouse tissues to study the efficacy of our gene therapy approach. To reduce the chance of mice developing tumours we will perform humane killing at 3 months for the majority of animals. Few mice will be kept for long-term studies for correction of FRDA. These mice will be continuously monitored for signs of ataxia and cardiomyopathy. Tumour development will be monitored by palpation and by regularly measuring animal weight in addition to observing their general appearance and wellbeing. These mice will also be subjected to a series of behavioural tests to assess their wellbeing. Any animals suspected of developing sickness will be humanely killed before suffering occurs. We also have previous experience in injecting gene therapy vectors and cells into mice and have published peer-reviewed articles describing this work. We will be provided with further training by collaborators. This further refines our use of animals by reducing wastage through exploratory techniques. We also have previous experience to apply our research to Objective 2 that aims to understand what vectors cause liver tumours in mice and the mechanisms that are behind this.

	We have shown that gene therapy vectors can provide correction of genetic disease. However they can also induce tumour development at previously determined time points. We use non-genetically altered mice that do not suffer from a genetic disease with virtually zero background of spontaneous liver tumour development. We will use suitable general anaesthesia and analgesia, where needed, to ensure minimal pain and suffering. Newly born mice will be anaesthetised using the latest methods that give good recovery with minimal suffering. We will be monitoring animals treated with gene therapy vectors and use vectors that carry a luminescence-generating gene that can be used to identify tumours without surgery via imaging technologies that will enable early time point humane killing to prevent suffering. Mice will also be monitored for tumours by palpation in case the luminescence gene is not active for any reason. Animals will be provided with an enriched environment that is best suited to their welfare and recovery under the guidance and advice of the named animal welfare officer and named veterinary surgeon. Mice will be placed in cages of between 4-5 per cage unless they are being bred as one male with 1-2 females. Overcrowding will be avoided in line with IACUC guidelines. Our observations from pilot studies or larger studies that we have used based on power calculations to reduce animal numbers will be used to ensure the appropriate severity limits always apply throughout our research. We might need to use strains of genetically altered animals in addition to those listed within this application, as and when they become available. Provided these strains fall within a mild-moderate band of severity and are justified by existing objectives. We will not seek authority on an individual strain basis. Strains that fall within alternative bands or contribute to additional objectives will be individually justified and a licence amendment sought. We will keep records of the genotype and phenotype of all strains used or generated.

Project 2	**Evaluation of novel anticancer agents**	
Key Words (max. 5 words)	Evaluation, novel, anticancer, agents	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	✓	Basic research
	✓	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	✓	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall aim of this project is to identify novel anti-cancer agents offering greater efficacy with improved toxicity compared with currently available therapies. Compounds are tested for adverse events to define the maximum tolerated dose (MTD) and their ability to inhibit the growth of primary tumours and metastases.	
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)?	The assessment of efficacy in an intact mammalian system is very important in selecting successful anti-cancer molecules. These development candidates should be highly effective with fewer side effects than current therapies resulting in a reduction in cancer mortality rates and/or improvements in quality of life of patients.	
What species and approximate numbers of animals do you expect to use over what period of time?	54500 mice 10750 rats These are maximum numbers to be used over 5 years.	
In the context of what you propose to do to the animals,	The majority of cancer studies used in this Licence are well tolerated by rodents. Mice and rats have	

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	been chosen because the growth and response rates of many different cancers have been well characterised and documented in these animals. It is not expected that serious adverse effects will occur but any side effects are likely to involve body weight loss and deterioration in clinical signs. Any animals exhibiting such signs will be removed humanely from the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animal tumour models are required to assess the effect of a test compound on tumour growth (efficacy). Cell assays can give a good indication of the potential anti-tumour efficacy of a compound but they cannot reliably predict *in vivo* efficacy due to differences in the growth patterns of cells *in vitro* compared to that *in vivo*, In addition, the PK/PD relationship, driven by distribution, metabolism and elimination, cannot be accurately modelled *in vitro*. *In vivo* techniques more closely mimic the clinical situation, in which compounds are eventually targeted. It is, therefore, essential that compounds be tested in a more clinically relevant environment. Currently, no *in vitro* alternatives fulfil the criteria required for cancer therapy. The failure of *in vitro* cell culture models to recapitulate key features of the tumour micro environment is a likely factor explaining why most new treatments have failed to provide clinical benefit. Finally, proven *in vivo* efficacy data is a prerequisite of the regulatory bodies who have the authority to approve or reject an Investigational New Drug (IND) application.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Protocols covered by this project licence application are designed to use the minimum number of animals possible. Tolerability studies are performed with small groups of animals in order to establish the maximum tolerated dose (MTD) and suitability of dosing regimen prior to larger efficacy studies. (Statistical analyses are not carried out at this point since this would necessitate larger sample sizes).

	Only then, can the larger and more complex *in vivo* efficacy studies commence in the knowledge that the animals are likely to tolerate the compound. Minimum group sizes for efficacy studies will be calculated using power analysis and will incorporate any previous experience of personnel or consultation with a statistician. The superficial location of subcutaneous tumours allows repeated calliper measurements, so changes in tumour volume can be measured directly over a period of time avoiding several cohorts of animals being sacrificed at different time points. A pilot study with fewer numbers of mice will be run when using new cell lines and therapies.
3. 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.	It is very well documented that rodents, especially mice and rats, are extremely useful for the pharmacological evaluation of novel anti-cancer agents *in vivo* delivering robust, reproducible data and so it is often unnecessary to evaluate efficacy of new compounds in higher species. Work is closely monitored by the NACWO and named veterinarian to ensure maintenance of high standards of animal welfare. Clinical signs are noted daily and body weight is recorded. In addition to tolerability studies, pilot studies may be conducted in a small number of animals in order to refine the parameters and methodology for ensuing studies defining the risk/benefit ratio of each procedure to generate statistically significant data whilst causing the least adverse effects on the animals. Where orthotopic models do not allow direct calliper measurement, non-invasive imaging methods will be used to monitor tumour growth and responses to therapy, also avoiding unnecessary sacrifice of animals. Optical imaging is a well-established platform for non-invasively tracking tumour cells within the animal in real time. In addition to using fewer animals, due to its sensitivity, it refines and shortens the study by producing data well before the animal is likely to show any signs of distress.

Project 3	**Regulatory mechanisms in normal haematopoiesis**	
Key Words (max. 5 words)	Stem cells, haematopoiesis, leukaemia	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We are trying to understand how blood stem cells operate and how they become perturbed in the context of leukaemia to form leukaemia stem cells which drive leukaemic disease and cause therapy resistance. We are also interested improving the practice of bone marrow transplantation for treatment of leukaemia and some of the side-effects associated with its use currently (e.g. anaemia and leukopaenia)	
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)?	From a scientific perspective, this project will offer insights into the mechanisms that allow blood stem cells to form blood, which is poorly characterized. From a clinical perspective, our project may improve clinical bone marrow transplantation and inform targeting of leukaemia stem cells to improve therapy for an otherwise difficult to treat disease.	
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, 6000 will be used over 5 years.	

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 expected severity level will be moderate. We will examine the growth of normal blood stem cells or leukaemic stem cells by intravenous injection of these cells into mice. The mice will be analysed for blood cell activity or leukaemia development at the end point. The main harm will be the possible development of leukaemia in select experiments where we are examining leukaemic stem cell function. The harm to mice in other settings where we are looking at normal blood stem cell function will be minimal and transient.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In many cases we use in vitro surrogates to model stem cell behaviour. In some instances however the use of animals is currently unavoidable as many facets of stem cell and leukaemia biology are only apparent in the context of the complex in vivo systems in which these cells and diseases naturally occur.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In the design of all our mouse experiments we strive to use the minimum number of animals that is commensurate with obtaining a robust and reliable result. We will apply optimal experimental designs and statistical analysis as key means of achieving reduction. I have 18 years of experience with this.
3. 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 the most frequently used model system to study biology of stem cells and cancer. Mouse and human haematopoietic stem and progenitor cells share conserved regulatory pathways at various stages in blood and leukaemia development. Animals exhibiting any unexpected harmful phenotype will be humanely culled under Schedule 1, or in the case of individual animals of particular scientific interest, advice will promptly be sought from the local Home Office Inspector and the Named Veterinary Surgeon. All the work involving mice with leukaemia will strictly adhere to the UK Coordinating Committee on Cancer Research (UKCCCR) Guidelines for the Welfare of Animals in Experimental Neoplasia.

Project 4	**Development of new biological anticancer agents**	
Key Words (max. 5 words)	Cancer, virotherapy, targeted, biologic	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Cancer is now the main single cause of death in the Western World, with over a third of people currently expected to die from this disease, Current treatments have Improved prognosis but had little Impact on cure rates, at least for the common types of cancer. We now aim to develop biological treatments that can have a dramatic impact on cancer treatment and improve cancer survival rates for common cancer types. We aim to develop a new type of cancer therapy based on cancer-killing ‘oncolytic’ viruses that may be given by intravenous delivery to treat metastatic cancer. As part of this, we aim to define factors that limit the ability of therapeutic viruses to replicate and spread through solid tumour tissues. Current clinical trials show that inadequate spread of viruses through tumours can be a limiting challenge for the success of oncolytic viruses. We will also develop armed’ oncolytic viruses capable of targeted expression of therapeutic biologics within cancer, leading to high local concentrations and minimising systemic toxicities Finally, we will assess whether other micro-organisms (such as bacteriathat are normally found in the gut) may also be useful for targeted cancer therapy.	
What are the potential	Oncolytic viruses promise very potent new cancer	

benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	therapies that improve on state of the art in two ways — they are more cancer- selective than most anticancer agents, and they are more powerful than most anticancer agents. The first oncolytic virus received its product licence in the USA in 2015, and now we are trying to improve on that agent to develop viruses that can be used to treat advanced cancer of various types.
What species and approximate numbers of animals do you expect to use over what period of time?	All of our work will be performed in mice. Over a period of 5 years we would expect to use up to 7000 mice. This number of animals is necessary to ensure our experiments are done properly and reach statistically significant results.
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?	Animals we use should not experience obvious pain or discomfort. We take care that animals are monitored regularly and carefully looked after. If any animal Is found to be suffering it will be immediately killed using a humane technique. Many of our mice will be used to grow subcutaneous or metastatic human tumours, but this is carefully monitored and the animals seem generally not bothered by the tumour (the experiment is terminated before the tumour reaches an excessive size). Nevertheless, if the growing tumour causes any distress to the animal it will be humanely killed at once. Animals also receive injections in the way normal human patients would. This appears to cause the mice no discomfort and they behave perfectly normally immediately afterwards. They are all humanely killed at the end of the experiment, and normally before they undergo any obvious suffering or show any signs of distress.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our work exploits'the complex cancer 'microenvironment' — including many non-cancer cell types that appear to support tumour growth. Some aspects of this also involve the innate and adaptive immune system, and these features cannot be properly modelled in vitro. Nevertheless we do make extensive use of cells in vitro and human tumour biopsies, direct from the cancer surgery, to explore the possibility of using non-mouse alternatives. The majority of our work is using cellular systems, and animals are used only for key experiments.
2. Reduction Explain how you will assure	Experiments are designed carefully, using pilot studies to estimate data variability, to achieve statistically-significant scientific outcomes using minimum animal

the use of minimum numbers of animals	numbers. We also make extensive use of in vitro models (both cell lines and primary cells obtained from clinical biopsy samples_ to try and minimise the numbers of animals we need to use.
3. 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.	Mice are the animal species of lowest neurological sensitivity that can support the complex tumour architecture and microenvironment similar to human disease. We make extensive use of imaging approaches to maximise the amount of information we can obtain from each animal, without subjecting itto any invasive procedures. Imaging readouts provide an important technological advance, because individual animals can be imaged at different times, mirlimising variation due to the use of different animals at different times, and provide much better quality data (with far less variation) than could be achieved before such techniques were available. Imaging techniques also allow us to determine when tumour growth is significant, allowing animals to be humanely killed before they experience any pain or discomfort from occult tumour growth. Animals are individually well looked after, and any animal in distress will receive professional veterinary attention to help It recover or, if recovery is unlikely, it will be humanely killed. In addition we apply the principle that it is better to subject multiple animals to a low level intervention than to subject a single animal to a distressing intervention, and this ensures that animal welfare is a top priority for us. Animal suffering will be minimised by ensuring they are subjected to the minimum disturbance possible, and by close liaison with the Named Veterinary Officer and use of pain relief (eg. analgesics).

Project 5	**Evaluating New Cancer Therapies**	
Key Words (max. 5 words)	Lung Cancer Models, Drug Discovery	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	✓	Basic research
	✓	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	✓	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We are trying to find new, effective treatments for lung cancer, a disease which kills more that 1.6 million people worldwide each year. We are trying two approaches. Mutations occur in lung cancer cells that allow them to grow indefinitely, and escape normal tissue control. So in the first approach we are trying to find drugs that specifically inhibit mutated proteins found only in lung cancer cells. Secondly, radiotherapy is used to treat lung cancer but it is not completely effective for many patients. So in the second approach we are investigating novel ways to improve the effectiveness of radiation with the aim of improving overall cure rates in cancer, and improving outcomes for patients with metastatic disease.	
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)?	The main benefits will be identifying potential new treatments for patients with lung cancer. The work done under this license will provide (i) the scientific rationale to start clinical trials, (ii) the guidance on which patients might benefit from new agents and how to combine the new agents with treatments already in the clinic, and (iii) biomarkers to select patients and determine whether the new treatment is actually working as planned	
What species and approximate numbers of	The animals used in this project are mice, because these provide the best models of human disease.	

animals do you expect to use over what period of time?	We expect to uses between 4000 – 5000 mice over the 5 year project
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 majority of mice will have tumours either implanted from cell lines or induced to develop by chemical or genetic means. Most mice will have no additional adverse effects other than transient weight loss. Because we are testing new medicines, both alone and in combination with chemotherapy and radiation therapy both, there are occasions when more significant adverse events can occur, such as skin reddening, diarrhoea, or the development of ulcers. However, these adverse events are rare (<5% of animals treated) and are most commonly seen when we first test a new compound. Therefore, we take great care when first testing compounds to make sure each animal is fully recovered before the next dosing to ensure the risk of any adverse effects is kept to a minimum. Because the mouse lung cancer models we use have parallels with human disease, the symptoms that animals with tumours develop also have parallels with human disease. Therefore, mice with lung tumours may show signs of laboured breathing, and mice with brain metastases may show neurological symptoms and so we take great care to ensure that these adverse effects do not become severe. Although at the end of each study all the mice are killed, we do extract the tumour cells and study them in culture (ex vivo) to identify what molecular changes have occurred during treatment to help guide our future studies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We do a substantial amount of preliminary work by growing cancer cells in culture to check that our biological hypothesis is strong. However, we cannot adequately model drug effects in animals in cell culture. This is because there are many different cell types in the tissues of animals that in turn affect the tumour response. In addition, the body alters the chemical nature of drugs (metabolism) in a way which is difficult to predict from cell culture studies, but which is essential to know if we are considering taking these drugs into patients.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We assess the variability of each animal model and use this information to calculate the minimal number of animals that would be needed to show reliably what the effect of the treatment we are testing will be.

	Also, wherever possible, we assess non-superficial tumour growth using imaging which markedly reduces the number of animals required in each study.
3. 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.	Mouse models of human disease are used since we know what results are needed in these models to take our findings into clinical trials. We always perform pilot experiments on unfamiliar tumour models and establish humane endpoints (where we end the experiment before any adverse effects develop) prior undertaking larger-scale experiments. Experimental animals are monitored daily for clinical signs of adverse effects. All animals undergoing surgery are given post-operative analgesia (pain relief). The frequency of monitoring is increased at critical periods during procedures and at those points where an animal's condition might deteriorate to minimise the potential for suffering. We do not work with tumour models that have a propensity to ulcerate, nor models that induce rapid disease-related weight loss (cachexia). Immune-compromised mice are maintained in IVCs under barrier conditions to avoid infections

Project 6	**Thermosensitive nanoparticles for cancer therapy**	
Key Words (max. 5 words)	Cancer treatment, drug development, liposomes, theranostics	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Many potential anticancer drugs fail due to problems with toxicity or inability to effectively get to the target. We are developing the use of Image guided drug delivery to enhance both the tumour uptake of anticancer therapeutics and to trigger the localised release of drugs. Several imaging techniques are used to assess how the drugs are dispersed through the body and how best to control localisation.	
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)?	Understanding how to localise anticancer drugs specifically to tumours enhances their effectiveness while better controlling their side effects. It may also allow the deployment of drugs that are otherwise too toxic to use andJor enhance the effectiveness of existing therapies.	
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use up to 500 mice over a period of 5 years.	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of	These studies involve the induction of tumours at the flank or the mammary fat pad of mice, followed by their treatment with drug or drug/nanoparticles combinations and hyperthermia locally applied on the tumour to increase local drug concentration. These tumours are	

severity? What will happen to the animals at the end?	maintained for up to 8 weeks but cause little distress to the animal. Drug injections, treatments and imaging are carried out under anaesthesia. Adverse effects- include limited weight loss (less than 15%) depending on the chemotherapeutic drug used, For MDA-MB23 1 breast cancer models metastasis could occur in the lung the earliest week post tumour induction this can lead to breathing difficulty and if this occurs the animal will be humanely killed. At the end of the study or if adverse effects indicate that an animal has reached the humane endpoint of the experiment it will be placed under anaesthesia and then humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Tumour growth and the interaction of anticancer drugs and hyperthermia is complex and cannot currently be studied without the use of animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	As much as possible the drug formulations are assessed and quality controlled using lab assays without the use of animals. The animal studies are combined with a variety of non-invasive imaging techniques (MRI, fluorescence, SPECT/PET, MW) to collect additional information on drug distribution and behaviour in the body. This additional information allows us to optimise ultrasound conditions and reduces the number of animals needed to assess the treatment's efficacy.
3. 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.	This study will use immunocompromised mice. These lack functional components of the immune system and so will not reject human cell line tumour grafts. This makes them a highly suitable model for these studies but requires the use of controlled environments to avoid infections. They also lack fur, which greatly eases imaging and ultrasound treatment. Using these animals adds technical complexity during their handling but significantly increases the clinical relevance of the results. All animals will be checked regularly for their weight, tumour sizes and for signs of discomfort or stress. All animals will be humanely killed at end of the study or before if adverse effects occur.

Project 7	**Mouse models of pancreatic cancer and therapy**	
Key Words (max. 5 words)	Pancreatic Cancer, Treatment, Tumour stroma	
Expected duration of the project (yrs)	5 yrs	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objective of this proposal is to investigate how tumour cells interact with host cells (the tumour stromal cells) and co-opt these to promote tumour growth, metastasis and therapeutic resistance.	
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)?	This project will contribute to the understanding of tumour progression and metastasis mechanisms. Moreover we will obtain new insight into the mechanisms whereby the tumour stroma promotes tumour progression and therapeutic resistance. Understanding these mechanisms may lead to the identification of new targets that can be used to develop novel treatment strategies. This project is specifically focussed on Pancreatic Cancer, but interactions between cancer cells and host cells is a common theme across many cancer types and as such novel insight may be applicable across other diseases.	
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use up to 3500 mice over 5 years for this project.	
In the context of what you propose to do to the animals,	Animals will be bred to show predisposition to pancreatic cancer or will receive a transplant of	

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	tumour tissue or cells from mouse or human cancer. Animals will be predisposed to cancer and will be monitored carefully for clinical symptoms. Symptoms include weight loss, swelling of the abdomen and development of visible or palpable tumours. Carefully trained staff will monitor mice with tumours and if the tumours interfere with normal behaviour, become larger than allowed by guidelines, or have any consequence greater than allowed by guidelines, mice will be humanely killed and the tissues will be analysed. Tumour cells will be grown in the laboratory. In some cases, we will treat animals with experimental chemical compounds and measure the effects on tumour growth or spread. This may involve adding substances to the food or drink or injection of substances. All animals receiving treatments will be monitored closely and any animals that display signs of being unwell, such as ruffling of the coat, reluctance to eat or move, weight loss of 20% or more will be humanely killed. At the end of the study, all animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Pancreatic Ductal Adenocarcinoma (PDAC) is a complex disease that consists of both tumour and non-tumour (host) cells. Moreover, the tumour contains an abundant extracellular matrix, secreted by the tumour and host cells. Together, this creates an unique microenvironment that modulate tumour progression and response to therapy. Due to this complexity there is no alternative model, which fully recapitulates PDAC. As such, any observation made *in vitro* needs to be validated *in vivo.*
2. Reduction Explain how you will assure the use of minimum numbers of animals	We aim to limit the number of animals used through several means: A) Extensive use in vitro models inform our in vivo experiments; B) all experiments are statistically evaluated prior to execution to ensure the correct number of animals are used and if needed we may perform pilot experiments to estimate the treatment effect and to ensure no adverse effects are observed from therapeutic agents; C) all experiments are appropriately analysed and evaluated following execution to ensure data are of high quality and D) where needed we will consult with colleagues and statisticians. We also constantly optimise our breeding strategies to minimise the number of animals needed to achieve the desired genotypes for our studies and we use tumour transplant models where appropriate, which do not require breeding of

	genetically altered animals and thus use fewer animals in total per study.
3. 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.	Mouse models that we are currently using faithfully recapitulate the human disease. We constantly work to improve husbandry and procedures, which minimize actual or potential pain, suffering, distress or lasting harm and/or improve animal welfare in situations where the use of animals is unavoidable. We ensure to provide the appropriate anaesthetic and analgesic regimes as well as appropriate humane methods of culling within animal facility. Environmental enrichment of cages housing the mice, with nesting material and tubes. Moreover through a continuous evaluation and comparison between our *in vitro* and *in vivo* models we aim to identify which aspect of the disease that may be faithfully modelled *in vitro*.

Project 8	**Anticancer Drug Discovery and Target Validation**	
Key Words (max. 5 words)	Anticancer, drug, discovery, target, validation	
Expected duration of the project (yrs)		
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objective of the programme is to develop new cancer treatments evaluating 5-50 potential new drugs in animal models in detail in order to identify 3-5 new cancer drugs for clinical evaluation. Cancer is the major cause of death from disease in the United Kingdom with approximately 150,000 deaths each year. Despite improvements in early diagnosis, the majority of patients present with disseminated (metastatic) disease and cannot be cured by surgery and local radiotherapy. Drug therapy (chemotherapy) is currently the only modality with curative activity against a wide range of tumour types and also has significant palliative effects in multiple common tumour types. The majority of patients are not cured by currently available drugs and there is an urgent need to develop new chemotherapies with significantly greater activity than those currently available.	
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)?	The major benefit of the programme will be the identification of new cancer drugs and improved understanding of the disease, generating additional possible avenues for the prevention, early diagnosis and treatment of what is the major cause of death from disease in most developed countries.	

What species and approximate numbers of animals do you expect to use over what period of time?	The project will predominantly use mice but some rats may be required. The estimated number would be approximately 10,000 mice and 550 rats over the five years of the project.
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?	We do not anticipate adverse effects to be a major issue using the techniques and checks we employ. The biggest risk factor is probably the risk of infection, especially after surgery, because the animals used do not have a fully functional immune system.. However, many precautions are used to maintain a sterile environment and infections have not been an issue over the past 5 years. The second biggest risk is the toxicity of new drugs. These cannot be predicted; however, by designing our studies well we have shown over the last 20 years that the number of animals showing severe illness can be minimised with < 10% of animals treated in toxicity studies being so ill they need to be killed and acute toxic death being very rare < 1%. Because of the measures we have put in place we would expect the project's level of severity to be moderate. At the end of all studies the animals are humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Use of animals is needed to address not only the issue of the therapeutic index of a new agent, i.e. the ratio of a toxic to a therapeutic dose, but also whole body pharmacokinetics and pharmacodynamics which *in vitro* models cannot currently predict (e.g. tumour microenvironment and vasculature can have a profound effect on the activity of a drug *in vivo* which would not be evident *in vitro*). In order to exploit targets for clinical development we need to study well defined human cancers, first *in vitro* and then in *in vivo* in a mammalian setting, where we can interrogate drug exposure, tolerability and therapeutic activity.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Only after the above rigorous *in vitro* testing are animal experiments considered, and animals are never used as a random screen. In addition, all experiments are designed to use the minimum number of animals to generate a statistically valid result.

3. 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.	We routinely use rodent models that have been consistently shown to provide a robust back ground for the development of new therapies in cancer research. These model have been developed over numerous years to be predictable in terms of the effects on the welfare of the animals and cause minimal suffering. Some of the newer, more biologically relevant, orthotopic models are more challenging to monitor and their growth can be more unpredictable. Our procedures are designed with animal welfare at the forefront to minimise the occurrence of suffering and distress to the animal. To which end we are collaborating in order to assess any pain caused by experimental tumours and facilitate the appropriate use of analgesia.

Project 9	**Modelling bone-tumour interactions in metastasis**	
Key Words (max. 5 words)	metastasis, breast/prostate cancer, myeloma, bone	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The prime objective of this project is to identify and characterize the cells and mechanisms responsible for the formation of metastases (tumour spread) in the skeleton and determine their dependencies so that they can be targeted and suppressed/killed.	
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)?	The project will provide novel information defining the processes involved in the development of metastases in breast and prostate cancer and the growth of myeloma in bone. The ultimate beneficiaries of this work are cancer patients who are at risk of the development of bone metastases. The project will provide markers to identify those at risk as well as features/processes associated with metastatic tumour cells that can be targeted by therapies.	
What species and approximate numbers of animals do you expect to use over what period of time?	The project will use a maximum of 10,250 mice over the five year period.	
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 severity will never exceed moderate for any procedure/protocol. Immunodeficient mice will be used throughout the project. In some studies standard laboratory mouse strains will be used. Genetically altered animals will be used where these do not show adverse effects due to the alteration. Some animals will be injected with tumour cells and tumours allowed to form in some but not all animals. The size of growing tumours will be carefully monitored and not allowed to significantly affect the	

	well being of animals. The focus is on understanding very early growth of tumours, not late stage disease with large tumour burdens. Some animals will be treated with drugs, radiation or other substances to test their effects on tumour growth and / or normal bone. Some mice will be used under non-recovery anaesthesia in order to produce blood cells for use in in-vitro experiments. All animals will be humanely killed at the end of experiments
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Modelling the whole interaction between tumour cells and the environments in which they grow in patients is not possible in cell culture. The interventions and procedures needed to understand these interactions cannot be used in patients themselves. Where possible when the effects of agents on single populations of cells (tumour cells, osteoblasts or osteoclasts) are evaluated, this will be done *in vitro*.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The systems we are using have been used in previous studies and the power calculations made define the minimum numbers of animals required to obtain significant data. Where possible fixed, histological samples will be shared with other projects –this is common practice within our laboratory
3. 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 animals used are specifically bred to not reject human tumour cells when implanted into them and in some cases genetically engineered to provide alternative environments for tumour cells to find out what is required for tumour growth/survival. The tumour models have been engineered so that their growth can be monitored *in vivo*, removing the need to cull cohorts at time points to measure effects. We have installed state of the art in vivo monitoring equipment to facilitate this. The aim is to study the very early stages in the development of tumours in a common site of secondary growth: the skeleton. Because we are studying early stages, tumours will not be allowed to grow large or cause distress to the animals and the drug regimes under consideration aim to alter animal physiology to make bone less amenable to tumour colonization and are of low toxicity.

Project 10	**Novel Biologicals for Tumour Immunotherapy**	
Key Words (max. 5 words)	Tumour Immunotherapy; Antibodies; Adjuvants; Targeted Delivery	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Novel types of treatment that make the immune system kill tumour cells have been identified and have proved very promising. However, these novel treatments still have to be optimised to improve their effectiveness. Our aim is to study and refine treatment strategies which will improve the ability of the immune system to kill tumour cells. The treatment strategies we will study involve delivering the drug to particular cell types within the body using molecules called antibodies.	
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)?	The potential benefits are more effective treatment strategies for the therapy of cancer patients and/or less severe side effects.	
What species and approximate numbers of animals do you expect to use over what period of time?	We will use up to a maximum 3,000 mice over the course of 5 years (approx. 600 animals per year).	
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?	Possible unwanted effects of experiments involving the growth of tumours in mice include weight loss, tumours affecting normal activity and bleeding from tumours. However, the likelihood of any of these adverse effects occurring is very low since animals will checked regularly and tumour size measured: mice will be humanely killed when tumours reach a specific size or when the overall well-being of the animals deteriorates to unacceptable levels. The expected overall level of severity will not exceed	

	moderate: for the majority of experiments we will not inject tumour cells into the mice (immunisation studies) and we expect the actual severity for these studies will not exceed mild levels. All animals will be humanely killed at the end of studies, or once they have been used for breeding and reached the end of their fertility.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The interactions between the immune system and cancer cells are complex and take place at various locations in the body. It is impossible to investigate this with isolated cells in cell cultures. Also, in order to test whether a novel treatment is effective and leads to the eradication of the cancer by the immune system, the new treatment method has to be tested in a living organism. Mice are best suited for these types of studies since their immune system is very similar to that of humans and since there are a lot of tools available that allow us to investigate the immune system in mice.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Whenever possible, we will investigate particular aspects of the immune response in cell culture tests. We only will test novel treatment strategies in mice once the results from the culture tests have convinced us that the treatment should work. We will use the minimal number of mice required to achieve conclusive results.
3. 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.	Mice are optimal for studying the immune system and the interaction between the immune system and tumours, since the mouse and the human immune system are similar and since a lot of experimental tools exist for studying immune responses in the mouse. We will reduce the suffering of the mice by monitoring their general wellbeing. Should any animals show signs of unacceptable distress, these mice will be humanely killed. We will develop tumour cells which produce so-called “reporter molecules” that will allow us to monitor tumour growth within the animals non-invasively, and without having to sacrifice the mice. Monitoring tumour growth in this way will reduce the overall number of mice that we will have to use in our experiments.

Project 11	**Signal transduction in lung cancer pathogenesis**	
Key Words (max. 5 words)	Lung, Cancer, Autophagy, NF-κB, Therapy	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Cancers that start in the cells lining the lungs are very common and hard to treat. Cancers form because of aberrant activity of cell signalling 'pathways', i.e. groups of proteins that instruct cells how to behave. Two important cell signalling pathways that lung cancers might rely on to continue growing are the so-called "autophagy" and "NF-κB" pathways. These maintain the health of cancer cells in the tumour environment. We need to identify proteins in these pathways and ask if removing their function in lung tumours will indeed reduce tumour growth. This project proposes to firstly identify such proteins using lung cancer cells cultured in the laboratory. Then it is proposed that we address whether these proteins play a role in mouse models of lung cancer, thus potentially validating these proteins as therapeutic "targets" for lung cancer. We will do this by disrupting the activity of test genes encoding these signalling proteins and asking *if* and *how* such actions affect tumour growth.	

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)?	By identifying proteins that drive progression of lung cancer, we will be able to improve our future approaches to treating this disease in humans. For example, we hope to identify proteins that might be good targets for inhibition by new drugs We don't propose such drug design on this project; this project will build the knowledgebase required for such translational activity. Thus, this work will constitute an important contribution toward the eventual diminishment of suffering and mortality from lung cancer.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use around 8600 mice over the 5 years.
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 majority of animals (8000) will be bred to sustain populations with the genetic modifications we require for the studies. These genetic modifications encompass those that give the mice lung cancer and those that disrupt the activity of test genes. By manipulating test gene function, thus removing a signalling protein from the mouse, it is possible we will affect the health of animals in some unexpected way, unrelated to analysis of tumour growth. However, this will be uncommon, as most manipulated genes will only lose their function in tumours (they are what are described as "conditional" genes – ostensibly normal until an initiating agent is used to switch them off only in tumours). We induce tumour formation in a number of mice, although these are in the minority (600). Most studies are completed before mice experience symptoms from tumours. However, in a minority of cases weight loss of a mild severity may be observed before the end of the experiment. Yet more occasionally, this can progress to greater, moderate severity weight loss or other moderate severity symptoms, such as breathlessness, anaemia and relative lack of mobility/loss of energy. All animals with lung tumours are killed at a defined time after the formation of tumours is initiated (or are killed immediately if moderate severity symptoms develop).
Application of the 3Rs	
1. Replacement State why you need to use	Tumours are complex structures and contain many different types of cells arranged in intricate patterns, communicating with each other and with the patient's

animals and why you cannot use non-animal alternatives	system. Testing in animals is the only situation where these complex tumours can form correctly and be meaningfully analysed. Thus far, this complexity has not been recapitulated in replacement systems using cultured cells.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use a dosing agent (inhaled virus) that provides some of the genes that are required for tumourigenesis rather than breeding different lines containing these genes together. This results in less unnecessary mouse breeding. Where appropriate, we use sophisticated, non-invasive and non-painful methods ("intravital imaging") to monitor tumour growth in animals – this can lead to better quality resolution over time of tumour growth. Dependent upon the experiment, this in turn may make it possible to replace killing mice at different time points, reducing overall numbers of mice that are given tumours. We are also very careful to use pilot experiments and/or known data on the rate and variance of tumour growth in these mice in planning final experiment sizes. Thusly, final numbers can be kept to a minimum while still permitting meaningful statistical analysis of the data.
3. 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.	Mice models of lung cancer have been developed over many years that carry various altered genes, which in turn lead to tumours forming in the lung that recapitulate the complex pathology of human tumours. No such physiologically-relevant models exist in other species. We use conditional genes to induce cancer that are in a 'latent' state and will be switched 'on' only in a small number of cells in the lining of the lung when the mice inhale a dosing agent. Thus we will breed mice with no adverse risk of tumour formation, either during breeding or in tissues outwith the lung. Similarly, where at all possible, the 'test' genes we use will be in such a 'latent' format and thus have no effect on mice, except in tumours (as desired). The information on tumour growth rate is obtained by analysis of tumour size at defined time points. By taking this approach, it becomes unnecessary to purposefully keep animals until they get overt clinical symptoms. We also have a good understanding, largely from our previous experience of the model, of the maximum time frames to perform these experiments to minimise the risk of such clinical indicators occurring while still obtaining scientifically useful data. Furthermore, we will also use any

	new information, including the latest analyses of our own results and analysis of the small pilot experiments we will perform prior to main experiments, to refine (shorten) time frames further if possible. Nonetheless, some mice in a group (<10%) will develop tumours more rapidly than the majority of similar mice, and thus may display symptoms before they are killed. We have a good understanding of the symptoms associated with such problems and have lots of experience monitoring for them. Indeed, we will implement a health monitoring and record-keeping plan that will allow us to identify moderate severity symptoms the day they occur. If these symptoms manifest, these small numbers of mice will be euthanised immediately.

Project 12	**Molecular imaging in cancer**	
Key Words (max. 5 words)	Molecular, imaging, cancer	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The primary focus of our work is early detection of treatment response with the aim of developing imaging methods that could be used in early phase clinical trials to get an indication of drug efficacy, and subsequently in the clinic to guide treatment in individual patients. We have also started to work on the more challenging problem of early disease detection and the development of imaging methods that potentially could be used for patient screening. We have been developing novel non-invasive imaging methods that can be used in the clinic to detect the presence of cancer, progression of the disease and the early responses of tumours to treatment.	
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)?	Translation of these techniques to the clinic should enable us to correctly diagnose cancer, and thus provide the appropriate treatment, and to determine whether a patient is responding to treatment within 24-48 hours, rather than have to wait weeks or months for evidence of tumour shrinkage, which is used currently as a measure of treatment response.	

What species and approximate numbers of animals do you expect to use over what period of time?	15000 mice and 700 rats
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?	Adverse effects relating to tumour establishment, development and the assessment of tumours and the administration of substances and sampling procedures are mild. Our severity limit is moderate. All tumour-bearing animals will be closely monitored and will be killed should clinical indications develop, such as loss of condition, a greater than 15% loss in normal body weight, significant abdominal distension, dyspnoea, digestive disturbances or neurological/behavioural abnormalities. Animals will also be killed if the tumour ulcerates or if tumour burden impedes any vital function (such as locomotion, vision, eating or excretion). In all cases, knowledge of the models will be used to guide health observations and to inform decisions on killing of animals before they become moribund. Animals will also be observed to best ensure the detection of tumour development at unexpected sites. At the end of experiment, all animals will be killed
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Any new imaging agent is first tested on cells growing in culture. Only if it is demonstrated to work in this simple system do we progress to experiments on animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have been developing a technique, termed ‘hyperpolarisation’, that increases sensitivity in the MRI experiment by more than l0,000x. The increased sensitivity has significantly minimised animal use. Also we have used power analysis to determine how many animals are needed to reach significance so as to minimise our animal use. We have used multiple non-invasive imaging modalities to obtain more information from fewer animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	Genetically engineered mouse tumour models are becoming increasingly important as systems in which to test new drugs and in which to develop new methods for detecting and predicting the responses of tumours to these treatments. This includes new non-invasive imaging methods, such as those that will be

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	developed in this project. The growth in the use of these models relates to our rapidly growing understanding of the genetic basis of cancer and our ability to genetically manipulate the mouse to produce accurate models of the human disease. We have optimised the procedures to minimise potential pain, suffering or distress, and enhance animal welfare.

Project 13	**Development of new anticancer therapies**	
Key Words (max. 5 words)	Cancer, Drugs, Translation, Biomarkers	
Expected duration of the project (yrs)	5 Years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The main objective of this project is to use mouse models of cancer to identify new drugs or combinations of drugs to treat patients. It is important that these treatments are safe, work well, and are administered to the correct patients. Research will be conducted to understand the way the drugs function and to develop tests that can tell doctors quickly whether the drug is working.	
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)?	We are an academic drug discovery group, and our aim is to develop drugs in types of cancers that are currently not investigated by the pharmaceutical industry. The research carried out in this project is likely to tell us whether new drugs will work in certain cancer patients. This would then allow doctors to test these drugs in patients in clinical trials, with the ultimate aim of finding drugs that can be approved and used to treat patients on a regular basis.	
What species and approximate numbers of animals do you expect to use over what period of time?	Studies will be performed in adult mice, using approximately 7050 over a 5 year period.	

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?	Continuous improvement in husbandry and experimental procedures minimize actual or potential pain, suffering, distress or lasting harm. The expected adverse effects for treatment of mice with anti-cancer drugs are weight loss, a change in normal behaviour and a loss of condition of their fur. The mice will be closely monitored by trained and competent scientists during administration of drugs to ensure that they do not go beyond a 'moderate' level of severity. In the unexpected event that they do suffer more than this, they will be humanely killed by trained staff. At the end of the experiment, all animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Cancer is a complicated disease, with cancer cells interacting with lots of other cells in the body, e.g. blood vessels. Drugs can also interact with other cells in the body, resulting in side-effects such as hair loss. Therefore, to obtain a true reflection of how a tumour and the patient is going to respond to a drug, it is important to perform the research in an animal and not in a test-tube. By undertaking this research in animals, it gives the best chance that the finding will be relevant when the drug is used in patients.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Before a large experiment is undertaken, a small experiment (pilot experiment) will be performed to ensure that the cancer model is growing properly and to estimate the minimum number of mice required for the full experiment to obtain reliable information. New technologies and techniques will allow the minimum number of animals to be used. For example, advances in blood analysis have enabled the analysis of drug levels from small blood samples (Microsampling). Therefore, a small sample can be taken from the same animal at different times, rather than a different animal being used each time, reducing the overall number of animals used. As can be seen from the diagram shown in the project plan section we also make extensive use of in vitro DMPK models prior to undertaking in vivo studies. This helps to ensure that we select the best compounds and our studies have the optimal chance of success.

3. 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.	Mice are selected as the species of choice as they are the industry standard for use as a cancer model. There is an enormous wealth of shared information and research in this species, which minimises the need for extensive additional research. The conditions under which experimental animals are kept are designed for the least possible disruption of natural behaviour and the highest possible quality of life. Continuous improvement in husbandry and experimental procedures minimize actual or potential pain, suffering, distress or lasting harm and/or improve animal welfare in situations in which the use of animals is unavoidable. The design of studies is constantly under review. Recommendations as to the refinement of experimental procedures will be assessed and implemented where a benefit is clearly seen. An example of this would be a recent change to pharmacodynamics studies, from treatment on an individual basis to treatment as a group. This has resulted in more robust data and a reduction in the number of animals required on study.

Project 14	**Mouse models of tumour growth and progression**	
Key Words (max. 5 words)	Cancer, metastasis, therapy, transplantation.	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Cancer, a major health issue worldwide, is a multistep disease resulting from a series of genetic mutations in genes referred to as oncogenes and tumour suppressors. Understanding how these genetic lesions change the normal cell to a cancerous one is vital if we are to prevent and treat cancer. Only in the context of the complete living animal can we fully understand how cancers develop, invade and spread to other organs. Using genetically modified (GM) mouse models with the same genetic mutations as in the human disease (so called 'patient-like' animal models) we can investigate the biological consequences of these lesions in cancer progression and identify those genetic events and signalling pathways which work together to drive invasion and metastasis. Such information will enable us to design new and targeted therapeutic approaches. The ultimate aim of the project is to use mouse models of human cancer in fundamental cancer biology research and in identifying new therapeutic targets. An important aspect to be studied is how the tumour environment influences the growth and progression of the disease. In addition, the impact of changes in cell shape as cells die on the inflammatory and immune responses will be studied, which may affect the way that tumours respond to	

	therapy.
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)?	This project will deepen our understanding of the underlying causes of cancer in general and of specific types of cancer which currently have a poor prognosis such as pancreatic cancer. Knowledge of the genetic causes will dramatically improve our ability to diagnose, treat and prevent cancer which affects one in three of the human population. We will also use mouse models to identify and test new therapies which will benefit cancer patients. This may involve finding novel ways to treat the disease, for example by altering the morphology of cancer cells as they die by apoptosis to increase their immunogenicity in order to induce inflammatory and immune responses.
What species and approximate numbers of animals do you expect to use over what period of time?	This project uses mice (including genetically engineered models). We expect to use up to 6,000 mice per year over 5 years. It should be noted that 70% of these will not undergo scientific procedures, but will be used solely for breeding and maintenance of colonies.
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?	Animals will be bred to achieve test subjects which may be predisposed to cancer. . Mice that do not show any adverse effects relating to their breeding and that do not undergo any procedures except for ear notching for identification and genetic testing will be humanely killed when they are no longer required for breeding. A proportion of animals will develop cancer because of their genetic makeup or because tumour cells have been implanted and allowed to grow. This may require administration of an inducing agent to switch on/off particular genes which only causes momentary discomfort but reduces off-target effects in other tissues. Animals will be monitored closely by highly trained staff for well-established clinical signs such as weight loss, swelling of the abdomen, and development of visible or palpable tumours. Some of these animals (up to 30%) will be given anti-cancer treatments or cancer causing agents (for example chemicals/irradiation) and the response to these treatments monitored. All animals on treatment will be closely monitored and may be blood sampled to follow changes in biomarkers which should cause only mild handling stress and momentary discomfort. Any animal that displays signs of illness such as weight loss of 20%, immobility or ruffling of the coat will be humanely

	killed. At the end of the study all animals will be humanely killed and tissues collected at post-mortem to gather as much information from the study as possible.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although many aspects of cancer research can be conducted using cells in the lab, it is not easy to fully model the complexities of a tumour which is an interaction of many different cell types (tumour cells, immune cells, blood vessels). Furthermore, the ability to monitor how cancer cells invade and spread to other organs (a process called metastasis) is very difficult to do other than in a mammalian model. Finally we know that cancer cells respond differently in the lab to anti-cancer therapies as they do in the context of the living organism and so testing the efficiency of such therapies requires a complete animal system.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We perform preliminary experiments using only a few animals, before scaling up to the appropriate numbers for a full study. Numbers are calculated based on our experience using the same models, published literature and advice of our in-house statistical experts. We also share animals between experimental groups where possible - e.g. when we need normal animals for controls, we can often obtain these from our breeding colonies where they would normally not be needed in a study. We constantly optimise our breeding strategies to minimise the number of animals needed to achieve the desired genotypes for our studies and we use tumour transplant models where appropriate, which do not require breeding of genetically altered animals and thus use fewer animals in total per study. To reduce numbers of experiments we also perform studies using cell lines or 3D models so that only our strongest hypotheses are tested in the mouse.
3. 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.	We use mouse models with the same genetic changes that are known to cause human cancer – so accurately replicating the human disease. These genetic changes are specifically altered in the tissue of interest so that unrelated effects in other tissues do not occur. All animals are monitored regularly for signs of normal behaviour and are humanely killed if they exhibit moderate adverse symptoms. All staff are expertly trained in these clinical signs. Regular monitoring of mouse welfare allows us to complete

	studies at the earliest endpoint in which we observe a significant result to prevent unnecessary suffering resulting from high tumour burden. We always refer to previous studies for adverse effects of anti-cancer therapies and when a group is given a treatment for the first time, we initiate the study with a small number of animals (n=3-6) which is closely monitored before extending to a larger number. Animals are housed in a dedicated facility proactive with environmental enrichment and receive anaesthesia and analgesia as appropriate.

Project 15	**In vivo imaging in cancer models**	
Key Words (max. 5 words)	Imaging, Cancer, diagnosis, therapy	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	In the UK, more than 330,000 patients are diagnosed with cancer annually and this is expected to rise to 425,000 by 2030. Further improvement in the management of cancer patients requires early detection of small tumours and cancer spread (metastasis). Treatment selection needs to be informed by individual patient and tumour type while monitoring of treatment outcome together with early detection of tumour recurrence in the individual patient is required to make evidence-based treatment management decisions faster and reliably. Imaging can non-invasively detect disease and measure treatment response. This project aims to develop and improve non-invasive imaging (via contrast agents and technologies) and to better understand the nature of cancers through application of imaging methods. The project is intended to discover new biology as well as deliver the missing links (i.e. animal data demonstrating contrast agent and technology capabilities) to start translation of our research into human trials.	
What are the potential benefits likely to derive from this project (how science	The project will enable us to develop novel imaging methods and techniques to better diagnose, understand and quantify the cancer development and	

could be advanced or humans or animals could benefit from the project)?	progression. It will allow us to develop and validate more efficient cancer treatments. It will have profound consequences to cancer treatment in that it will use quantitative and objective while non-invasive measures to (i) identify patients at high risk of cancer spread, (ii) provide appropriate treatment earlier, and (iii) better monitor treatment response. It will also save cost through avoiding debilitating and expensive treatments in patients who will not benefit.
What species and approximate numbers of animals do you expect to use over what period of time?	9,200 mice and 2,000 rats.
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 animals will develop cancer as a consequence of administering cancer cells to them. The majority of routes by which we give the animals cancer will cause only mild discomfort. Some methods require surgery while animals are unconscious (due to general anaesthesia). The development of tumours and their progression as required in this project will be the cause of moderate adverse affects including cancer spread. We will carefully and frequently monitor tumour growth in each individual animal including clinical markers such as, for example, appearance, indications of pain, weight and food intake. Imaging requires the animals to be still and often also requires contrast agent administration. We have previously optimized anaesthesia and will use safe ranges of contrast agents. Consequently, no adverse events are expected to be caused by imaging. For therapy we will use previously determined safe amounts wherever possible. Once experimental goals or predetermined humane endpoints are reached the animals will be humanely killed and tissues used for analysis and production of experimental data to answer scientific questions posed in this project.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	We need to use animals in our research because: (1) we need to demonstrate that our new methodologies and technologies work in animals

use non-animal alternatives	before they can be applied to humans; (2) certain aspects of tumour biology can only be studied in live animals, for example, what other cells enter the tumour at what time and with what consequences for tumour growth (the so-called tumour microenvironment), how and to where cancer cell spread, and tumour recurrence and treatment resistance; (3) contrast agent distribution requires whole organisms with intact physiological barriers and excretion mechanisms.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will perform preliminary experiments that do not involve animals to thoroughly characterize contrast agent candidates. Any contrast agent candidates that are unlikely to result in promising candidates for translation into humans will be eliminated at this stage and before testing in animals. Furthermore, all biological research will be based on solid scientific data/hypotheses including in vitro experiments as far as possible. When demonstrating capability for new instrumentation dedicated to cancer imaging, we will first ensure the instruments are working well, for example, by using a test environment. The use of non invasive imaging to determine the distribution of a novel contrast agent rather than killing at sequential time points (with removal of tissues for analysis) is a major contributor to reduction of required animal numbers. (1) Imaging allows repeated observations/measurements over a period of time ('longitudinal study') on the same animal, with humane killing only at the last time-point. Thus, if a longitudinal study involves six time-points, the numbers of animals are reduced to one sixth by use of repeated imaging. (2) In addition, since each animal serves as its own control to compare different time-points, the data obtained are statistically more robust. The result is that smaller animal cohort sizes are needed. These attributes of imaging contribute to a greatly improved benefit:cost ratio (benefit=data quality and quantity, cost=animal numbers, procedures and their severity).
3. Refinement	**Species:** Mice and rats are the species of least neurophysiological sensitivity that support the growth

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.	of human-derived tumours such that those tumours resemble the human situation (i.e. serve as a suitable model). Furthermore, mice and rats have a body size compatible with the requirements of the imaging technologies relevant for human use (e.g. instrument resolution, sensitivity etc). **Models:** Induced cancer models and cancer models based on genetically altered mice are required to study cancer imaging agents and cancer development/progression and therapy in an environment resembling the human situation. Isolated cancer cells are not suitable for this purpose as the impact of other cells and organs within the organism is not taken into account. We will use different cancer models as there is no single cancer model suitable to study all aspects of cancer. This is because different cancer types are effectively different diseases governed by varying biological features, which also evolve in different manners depending on how their tumour microenvironment is composed. Consequently, studying those cancers as well as developing specific new and imaging tools requires the use of different models and progression stages. We will use models that are most suitable for each objective; these include tumour models that mimic the intrinsic variety of human tumours in the presence or absence of an intact immune system, at differing progression stages, and with or without treatment. **Minimising suffering:** As a result of cancer cell administration to rodents they will develop tumours. Animals will be carefully monitored for tumour development and various clinical signs (e.g. pain, weight, food intake etc). The tumours will be allowed to grow to a size suitable for the respective experiment and limited by clinical signs of adverse effects (humane endpoints) or a maximum size (for superficial tumours). Animals will be humanely killed once any endpoint is reached or before if the experimental objectives have been reached. Inhalation anaesthesia will be used to minimise any associated transient pain and distress, for example, during tumour induction procedures and imaging. In addition, full recovery between periods of anaesthesia, rehydration during long imaging sessions, respiration/cardiac function monitoring, body temperature monitoring/maintenance will be

	performed and conducive to animal wellbeing. We will further combine imaging sessions wherever possible to reduce to the number of anaesthesia sessions per animal.

Project 16	**Cancer Therapy-Efficacy Licence**	
Key Words (max. 5 words)	Oncology, Tumour, Efficacy, Pharmacodynamic	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Whilst current cancer treatments provide some survival benefits (50% survive cancer for 10 years or more in 2010- 2011), they are often associated with significant side effects. Thus there is a clear need for improved and better tolerated medicines that can be used either alone or in combination with existing or other new therapies. The aim of this project is to develop therapies that reduce, inhibit or prevent the growth of tumours leading to new and improved cancer therapies. This will require the use of animal models, including models of tumour growth in rodents (mice and rats only) to help in the discovery and development of novel targeted medicines for the treatment of cancer. There are 6 specific objectives that will be used to achieve these aims: Objective 1 — To determine the efficacy of new candidate drugs on the growth of tumours Objective 2 — To determine the duration of activity of new candidate drugs in tumour models Objective 3 — To determine the development of	

	resistance mechanisms Objective 4 — To optimise treatment scheduling for clinical use Objective 5—To investigate mechanism of action using pharmacodynamic biomarkers Objective 6 — To determine the efficacy of new candidate drugs by assessing changes to normal physiological processes.
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)?	By using targeted therapy approaches, the treatments should be more effective and should have significantly reduced side effects than those associated with current therapies. This will significantly improve patient's quality of life and overall survival of cancer patients.
What species and approximate numbers of animals do you expect to use over what period of time?	Only rats and mice will be used on this project. Up to 125000 mice and 24,500 rats will be used over 5 years Approximately 90% of the total usage will be mice and "IO% will be rat usage.
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?	Adverse effects related to tumour inoculation may cause brief discomfort or pain. Adverse effects will be minimised by: • limiting volumes • choice of appropriate needle size • application of good technique by trained licensees The tumour types used are very well tolerated and only one superficial tumour will be used per animal. Tumour size and condition is monitored closely on a daily basis and we will use the least invasive tumour site/line that will achieve the scientific aims and will apply the earliest endpoints to meet the scientific requirement of the study. Animals will be culled if the tumour results in significant pain or distress. Clinical signs related to the pharmacological action of the compound may be seen and mild to moderate signs of toxicity are possible. Animals will be humanely killed if this persists. Local irritation at the site of injection may be observed. Animals will be closely observed on the day of dosing. Animals are observed by trained staff, with referral to the Named Animal Care

	and Welfare Officer, veterinary staff and Project Licence Holder as necessary. All animals will be regularly monitored for weight loss and general condition. Weight loss as a result of repeat anaesthesia may occur and this will be minimised by correct dosing, accurate weighing and good maintenance of body temperature during the period of anaesthesia and the recovery phase. Animals that are used where the immune-system is compromised will be housed in sterile conditions. The protocols are classified as moderate severity. Animals will be humanely culled at the end of the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Non-animal alternatives are used in the identification and selection of compounds and generally include measurements of the likely effect of the agent on the target cells. Activity in particular cell types however, cannot predict the likely in vivo activity given the complexity of issues such as bioavailability, metabolism and elaborate physiological interactions associated with tumourgenesis and therefore the whole animal is needed for the studies proposed in this licence.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To maximise the scientific integrity of data generated and to use the minimum number of animals, in house statistical expertise will be applied to all experimental design and analyses. Where plausible the following statistical guidelines will be used to minimise the number of animals required for each procedure: • meaningful biological change and measurable endpoints will be defined • estimates of biological variability will be used in sample size and power calculations • animals will be allocated in an optimal way based on estimates of biological variability established from accrued historical databases, pilot studies or published data. • regular monitoring and updating of biological databases with regular review of group sizes. • one-sided (rather than two-sided) statistical tests will

	be used wherever appropriate (e.g. when identifying inhibition rather than change) • statement of intended statistical analyses and justification for use, if any, of transformed data (e.g. tumour growth data may be analysed on the logarithmic scale if the variance of tumour measurements increases with the mean) • statistical power will be set to a minimum of 80% (e.g. at least an 80% chance of declaring the defined 'meaningful biological change' as being statistically significant) • multiple treated groups will be compared against one control to reduce the number of studies performed. Group sizes may be weighted to reflect this. For each and every experiment, as part of Good Laboratory Standards, an experimental protocol is written which includes: • a statement of the objective(s) • a description of the experiment, detailing experimental treatments, the size of the experiment (number of groups, number of animals per group), duration of experiment, scientific endpoint Every experiment conducted will have a Statistical Health Check associated with it which has been signed off by a statistician.
3. 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 overall plan of the work is to support the discovery and development of novel cancer therapies to benefit human health in the treatment of cancer. Only rats and mice including immune-deficient strains are used on this licence. Using non-mammalian species of lower neurophysiological sensitivity is not possible since they lack appropriate tissue physiology. Although exact replication of all pharmacokinetic parameters between species is not possible, many features of human PK can be predicted from those observed in small mammalian species unlike effects seen in lower organisms. The most appropriate species and strain of mice and/or rats will be chosen based on previous data that has been used to generate single agent efficacy data. Mice will be used in the majority of studies unless there is a

	scientifically relevant reason that mice cannot be used, for example, compound metabolism issue with the compound. The choice of strain will be driven by the choice of tumour model. For human tumour lines immune-deficient animals are required to support the growth of the tumour, the least immune-deficient strain required to promote good, reproducible tumour growth will be used. The optimal conditions for tumour growth will already have been developed. For anaesthetic protocols the optimal anaesthetic regime relevant for the species/strain will be developed and used in conjunction with the NVS. Where necessary, pain relief will be used under the guidance of the NVS.

Project 17	**Understanding and targeting the drivers of malignancy**	
Key Words (max. 5 words)	Cancer, prevention, treatment, metastasis	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	According to the latest statistics compiled by Cancer Research UK 338,623 people in the UK were diagnosed with cancer in 2012 and unfortunately there were 161,823 deaths from cancer in the same period (http://www.cancerresearchuk.org). There is therefore a need to understand better the key molecular determinants of cancer progression and devise new more effective treatments to benefit patients with cancer. This project aims to use different animal models to understand which pathways control tumour initiation, tumour growth at the primary site and the spread of tumours to distant sites within the body (metastasis). This will be achieved by manipulating expression of key proteins that we think are likely to influence tumour behaviour. Using this information we will then test potential new drugs and treatment modalities for their ability to prevent tumour initiation, growth and/or spread.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	Through this work we will make a significant contribution to our understanding of the basic biology governing tumour behaviour. In addition the project will provide added value to the translation of such findings into clinical benefit for patients with cancer by	

project)?	providing robust evidence to support the rationale clinical development of new drugs.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5 years we will use around 20000 mice to maintain our genetically modified mouse lines. Around 20000 mice will be used for experimental purposes.
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?	Many animals will develop tumours and in most cases these will not result in adverse effects with animals living quite normally without evidence of distress or discomfort. However in some case animals will have symptoms such as breathlessness, loss of weight or abdominal swelling, or reduced mobility, which is of a moderate severity at which time they will be killed. All other tumour bearing animals will be killed at pre-defined time points according to the experimental protocol at which time tissues will be taken for analysis. Other procedures that animals will undergo include surgery where the risk of death from anaesthesia is <1% and the risk from post-surgical infection is around 1%, and administration of substances including therapeutic agents through a tube into the stomach can result in damage to the throat.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Tumours are comprised of multiple cell types and their behaviour is governed by the surrounding environment in which they develop. Currently there are no non-animal laboratory models that recapitulate this complexity and it is therefore necessary to use animal models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Based on our experience we have devised the most effective breeding strategies to generate mice that will develop tumours therefore reducing the unnecessary breeding of mice. For experimental mice, experimental design will be kept as simple as practically possible, in order to maximise the information obtained from the minimum number of animals. This is based on statistical calculations to ensure experiments are sufficiently powered to generate significant results.
3. Refinement Explain the choice of species and why the animal model(s)	Mouse models of cancer have been chosen as these have been developed over a number of years and provide the best animal model in which to study cancer associated behaviour and response to

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.	therapy. Use of different models allows us to study the different aspects of tumour biology including tumour initiation, growth and metastatic spread. Advances in technology allows the specific expression of tumour initiating genes in only one organ (eg breast) thus minimising potentially harmful effects in other tissues. In addition such technology allows us to manipulate expression of certain proteins within tumours and determine their role in tumour behaviour. This provides a very flexible and powerful approach to understanding what drives tumour development. We will ensure that all animals receive the highest standard of care, and where appropriate social, environmental and behavioural enrichment will be provided. This is not possible in some cases where such enrichment may interfere with imaging devices implanted in the animals. Close monitoring of tumour development will ensure animal suffering is kept to a minimum. Our experience of these cancer models and the clinical signs has allowed us to refine the endpoints and in many cases animals are killed before clinical symptoms appear.

Project 18	**Regulation of Normal and Malignant Blood Cells**	
Key Words (max. 5 words)	Stem Cell, Leukaemia, microenvironment, niche, self-renewal	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The general goal of this research program is to understand mechanisms which control the normal blood and leukemic stem cell functions. The specific objectives of this proposal are: 1. To determine functions of candidate regulators of normal blood stem and progenitor cells; 2. To explore how genes found to be altered or mutated in human leukaemia contribute to the development of disease; 3. To determine factors/cells that constitute the stem cell niche and evaluate the role of these niche factors in the maintenance of normal and leukemic stem cells 4. To investigate chemoresistant leukaemic cells	
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 results will contribute both to an improved manipulation of blood stem cells for therapeutic use and to an understanding of blood cancer notably leukaemia development. Our studies may also identify novel therapeutic targets for improved treatments of leukaemia and potentially other forms of cancer. These studies should also provide insight into the regulation of blood stem cells, which reside in bone marrow and produce mature blood cells throughout an animal's	

	lifetime, Eventually, these results might be used to reduce the morbidity and mortality following bone marrow transplantation.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice only. We will produce up to 30,000 genetically altered mice over the 5-year period of the licence, 60% of which will be used in experiments. The remainder will be the animals necessary to breed those used in experiments. Wastage is minimised by careful planning of breeding, and, where possible, littermates with the relevant genotype are used as controls, such as crenegative or non-genetically modified from hetero/hetero crosses.
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 first part of the projects includes the breeding and use of transgenic mice. Tissues or cells from transgenic animals will be harvested and analysed in vitro using cell and molecular approaches to understand the functions of candidate regulators in normal blood stem and progenitor cells. The second part of our project includes experiments using human normal and leukemic cells and where it will be necessary to assess the behaviour of the cells also in vivo, as in vivo study still represents the gold standard assay for stem cell function. In this event, cells will be transplanted into recipient mice, a similar procedure as bone marrow transplantation in patients with leukaemia. We will monitor the engrafted cells to understand the development of normal or Leukaemia cells. Once the engraftment is established, we may then carry out a therapeutic treatment for these mice to study the characteristics and mechanisms of those chemoresistant cells. The transgenic mice we use in this project are riot expected to exhibit any significant harmful phenotype although many are likely to have some impairment of their immune system and may succumb to infections not affecting normal mice. They will be kept in pathogen free status within barrier systems to protect them from infections. Animals carrying transgenes, mutations or with transplantation may develop leukaemia. In this case leukaemia can be recognized by anaemia (reduced red blood cells, and bone marrow failure, similar symptoms observed in patients. Close observation of animals will be used to identify the onset of symptoms associated with a developing leukaemia burden. However, it is not possible to fully predict the nature or severity of any

	potential defect and for all types of mice there will be careful monitoring for possible side effects. Animals exhibiting any unexpected harmful phenotypes will be humanely killed, or in the case of individual animals of particular scientific interest, advice will be sought from the Named Animal Care & Welfare Officer (NACWO) and facility vet.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have explored the possibility of experimental methods that do not require the use of animals at all or do not require live animal experimentation. As a consequence, much of our research is carried out in culture with normal cells obtained from healthy human volunteers or culled mice. In order to replace animals we have developed recently a new in vitro model which allowed us to maintain human normal and leukaemic stem cells for at least three weeks. We will make use of this method to screen gene functions. Only for key genes we will use in vivo functionality to confirm effect on stem cells.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The majority of mice used in this project will be generated by breeding of transgenic animals. The smallest number of mice required for each experiment will always be applied. These numbers are based on past experience and on theoretical calculations. Another important means of achieving reduction will be to apply most efficient breeding strategies. We will replace breeders before their reproductive performance declines. Non-productive breeders will also be replaced. We will regulate the breeding depending on needs; if we predict that offspring from a particular litter will not be required for several months, we will adjust the number of breeders accordingly.
3. 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.	Mice are chosen because they are the lowest form of mammal available for study and are the most frequently used model system to study biology of stem cells and cancer. Other advantages of the mouse model include the availability of antibodies for the identification and purification of different classes of stem cells and mature blood cells and the availability of in vitro and in vivo functional assays. To ensure technical competence, the staff performing the experiments will be fully trained and directly supervised by myself or senior postdoctoral fellows who have extensive experience in experiments on animals. To minimise infections of

	immunocompromised mice, where appropriate, the animals will be housed in individually ventilated cages. Cages, food, water and bedding of immunodeficient animals will be sterilised. If mice are in pain, analgesic will be given. Transgenic animals exhibiting any unexpected harmful phenotype will be humanely culled, or in the case of individual animals of particular scientific interest, advice will promptly be sought from the local Home Office. Inspector and veterinarians. All the work involving mice with leukaemia will be undertaken in accordance with the principles set out in the National Cancer Research Institute (NCRI) Guidelines. Since human leukaemic cells will only grow in haematopoietic organs, the mice do not develop visible tumours and normally remain healthy. Some mice might develop mild anaemia, and br sign of bone marrow failure/ myelofibrosis. In rare cases, leukaemic cells might spread to different organs and form tumours. Experiments will be terminated in most cases before symptoms such as tumours or anaemia develop. In all our experiments we will set humane endpoints and write an experimental protocol, which will include details of possible adverse effects. 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.

Project 19	**Oncolytic HSV as an anti –cancer therapy**	
Key Words (max. 5 words)	Oncolytic , cancer therapy	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Oncolytic viruses are viruses that have been engineered to infect, replicate in and kill cancer cells specifically. This project aims to improve Herpes Simplex virus (HSV) based oncolytic viruses for use in cancer treatment. The objectives are to look at an existing virus that is already in the clinic in combination with a number of other cancer treatments in the hope that by using a combination approach it will be more successful in completely eradicating a tumor. The second objective is to test new improved viruses that have already been tested in the laboratory and shown improved efficacy in tumour cell killing.	
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)?	This testing will allow us to more fully understand existing therapeutics and will be able to help us make the decision about which new therapies work best and determine which ones to move forward to a clinical setting	
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice and less than 200 per year. Experiments are carefully planned and experiments refined in order to maximise the amount of data we can collect from each individual animal	
In the context of what you	The experiments in question are of moderate	

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?	severity. Small tumours will be established in the mice by a single injection under the skin that can be monitored easily. Tumours are not allowed to grow past a size that causes the animal pain, discomfort or mobility issues. All animals will be euthanized at the end of the experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are working on 3D tissue modelling but still require a small number of animals with intact immune systems in order to measure the effects of the therapy in an entire organism
2. Reduction Explain how you will assure the use of minimum numbers of animals	Advances such as imaging allow us to maximise the amount of data. Other advances such as FACS analysis- which allows us to determine what types of immunes cells are up and down regulated allow us to maximise the information we get ex vivo (from the cells we obtain for the animal after is euthanatised)
3. 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.	Mice are housed at licences research facilities that are fully staffed with technicians and vet staff. The well being of the animal is paramount and experiments will be discontinued if there are any suspected health problems.

Project 20	**Stem cell function in tissue regeneration, diabetes and cancer**	
Key Words (max. 5 words)	Stem cells, cancer, diabetes	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We work on two important diseases, namely diabetes and cancer. We use the best mouse models possible to understand the mechanisms of disease progression, with the ultimate aim to develop better therapies. Diabetes is a prevalent metabolic disease that affects over 2,5 Million patients in England alone (5.8 per cent of the population) whereas cancer is one the greatest cause of mortality world-wide. Understanding how these diseases develop is a pre-requisite for better treatment options. Survival rates and disease management have improved in the last decades, but there is clearly still a lot of progress to be made in the understanding of the diseases and in optimising treatment strategies. Our cancer studies focus on intestinal, pancreatic and lung cancer. We try to elucidate how these cancers develop, and identify weaknesses that are of therapeutic value. Our work on diabetes centres on the de-novo generation of beta cell. Beta cells produce insulin, and are lost in diabetes patients. We want to identify mechanisms that activate these cells and investigate how they can be regenerated in a diabetes patient. We address these questions at the molecular and cellular levels using cutting-edge	

	molecular biological technology.
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)?	We anticipate that the information gathered from work carried out under this Project Licence will make a valuable contribution to our general understanding of cancer and diabetes. Our studies on intestinal, pancreatic and lung cancer should help us to unpick the molecular and cellular mechanisms that occur during the course of cancer development and could be important in terms of the design of effective and long-lasting anti-tumour strategies. Furthermore, given the enormous therapeutic need for diabetes, we expect our studies to prove useful in the design of a potentially curative diabetes treatment.
What species and approximate numbers of animals do you expect to use over what period of time?	We will produce up to 75,000 genetically altered mice over the 5-year period of the licence. Due to the complex mouse genetics required to model human diseases, the desired phenotypes are obtained with relatively low frequency, and thus about 25% of the animals will be used in experiments. The remaining mice will be used to breed to maintain the colonies and produce experimental animals. Wastage is minimised by careful planning of breeding, and, where possible, littermates with the relevant genotype are used as controls, such as cre-negative or non-genetically modified from hetero/hetero crosses.
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?	We are careful in all our experiments to ensure that we use procedures that cause the least possible suffering. Some of our experiments involve surgical procedure to induced tissue damage, namely pancreatic duct ligation or pancreatectomy. The scientists performing the surgery are highly experienced, thereby keeping adverse effects to a minimum. If persistent lethargy or weight loss may occur, in which case they will be humanely euthanised. We would like to study the function of stem cells and tumour cells by transplantation. For kidney capsule transplantation and intra-pancreatic cell injections, mice will be anesthetised and cells will be transplanted. We have not experienced complications with such procedures during the period of the previous licence, but we would kill the operated animals should persistent lethargy or weight loss occur. In rare cases mice may undergo or bone marrow transplantation. Recipient mice will first be treated

	with low doses of ionizing radiation before reconstitution of the bone marrow. Also in this case, should signs of engraftment failure such as persistent lethargy or weight loss may occur, mice will be humanely euthanised. Overall, we anticipate that the vast majority of the animals used in our experiments will experience undetectable or mild side effects. In actuality no more than 5% should experience even moderate severity.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Diabetes and cancer are complex diseases, which involve the interplay of multiple cell types, including the immune system. These complex cellular interactions cannot be reproduced in cell culture systems, making animal experiments a necessity. My group follows the current scientific literature closely, in particular with regard to diabetes and cancer. We commonly employ literature searches and use the 3RS' protocol design resources (https://www.nc3rs.org.uk/experimental-design) to evaluate the relevance of the proposed animal models and to refine our research subjects. We closely interact with colleagues interested in similar research questions to actively prevent duplication of experiments. We try to make optimal use of preliminary experiments using conventional cell culture methodologies or, where at all possible, in vitro organoid cultures. Using this approach we will only consider animal experiments when we have already investigated our scientific question in great detail. However, even the most sophisticate cell culture systems are not able to reproduce the complex cellular interplay happening in diseased tissues, and therefore mouse experimentation is mandatory.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The use of preliminary cell culture studies means that for many of our experiments means we have a well-defined hypothesis before we embark on mouse studies. Therefore the scientific goals of the experiment are clear and we can perform statistical 'power' calculations to determine the number of mice required to give a satisfactory and statistically significant answer to our scientific question. Where possible, for example for tumour studies using complex genotypes, we derived ES cells harbouring all genetic modifications. These ES cells are then

	used to generate chimaeras, which harbour all necessary genetic alterations, and these chimaeras can be directly used to study the tumour type of choice. Thereby we obtain mice that already have the genetic alterations needed for our experiments, which means that many fewer mice are needed for breeding. We also try to maximise the information we get from each mouse. We ensure that multiple organs are sampled, for example for diabetic mice also blood is analysed, so to maximise the amount of useful information. In addition, we will analyse the same animal on more than one occasion we get a much clearer picture of how a disease proceeds, an example being the measurement of blood glucose levels over time in a diabetic mouse.
3. 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.	To understand the mechanisms and disease progression of diabetes and cancer, it is mandatory to use model organisms which have similar organs and cell types. This means that our studies require animals with similarly complex solid organs, such as the lung and the pancreas. It is also important that the cellular composition and the principle function of these organs and the cells contained within these organs are comparable to the human situation. Mice are the most suitable model organism to enable us to answer our scientific questions, because of the relative ease of husbandry, rather short generation time and relative ease of genetic manipulation. We are constantly aiming to refine our experimental procedures to minimise adverse effects. In close communication with the animal house staff, we strive to optimise animal welfare, diligently updating and refining our husbandry according to current best practice. At our institution, the mice are kept in modern facilities, using individually ventilated cages, access to light according to natural light/dark patterns, and to continuously available food and water. Trained staff regularly checks our mice, and the cage environmental is enriched to encourage and facilitate the natural behaviour. When mice undergo experimental procedures, we consider the potential effects on the animals' welfare and take appropriate steps, including extra checks, to avoid or minimise discomfort/distress. For all our experiments we ensure that we design our studies to elicit the fewest and least severe adverse effects

	possible. We commonly restrict a genetic modification only to a specific tissue or cell type, reducing the impact on the whole animal. We always strive to use littermates as controls as these animals will this have the same genetic background but do not carry the genetic alterations.

Project 21	**Modelling cancer predisposition by BRCA2 mutations**	
Key Words (max. 5 words)	Cancer; Genetic alteration; Pancreatic cancer	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The genetic material within cancer cells is highly unstable. The overall purpose of our work is to understand how this instability contributes to cancer growth, and to use this new knowledge to better pinpoint genetic risks for human cancer, and to develop new approaches for cancer detection and therapy. In particular, we will study pancreatic cancer, a leading cause of death, for which there is an urgent unmet medical need to improve detection and therapy.	
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 work will better define the key genetic and cellular events required for a tumour to progress. By studying this process, we expect that better clinical tests can be developed to identify early cancer growths before they develop into full-blown disease, and also new treatments can be developed to target these growths at an early stage. In particular, the models we have developed recapitulate key events in the progression of 10-50% of cancers of the breast, ovary, pancreas or prostate, which affect thousands of patients each year in the UK. Our work will particularly benefit patients suffering from pancreatic cancer (~9000 cases/year in the UK; ~37000 in the US), a leading cause of deaths. Currently, 80% of patients are inoperable at diagnosis, of whom only ~10% will survive for 5 years after diagnosis, so there	

	is an urgent need for new interventions.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse Approximately 35,925
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?	Most of our work involves the creation of genetically altered strains, which may experience mild alterations in growth or development. When interbred, some mice will develop pancreatic cancer and other internal tumours (e.g. lung cancer), and experience effects of moderate severity. We expect to test the effects of substances given to these mice on cancer growth and image the effects of substances on internal and external tumour growth, which may cause effects of moderate severity. Mice will be humanely sacrificed if they experience unexpected adverse effects or at the end of the work. At all times, we will take care to avoid unnecessary suffering.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Wherever possible, we will use in vitro experimental tools such as cell lines that we have developed, but cancer growth occurs in tissues, and involves multiple systems of the body, and so it cannot be modelled accurately in vitro. Moreover, non-animal alternatives that accurately model cancer growth, detection or treatment have not yet been developed.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To assure the minimum use of animals, we will: (a) develop genetically altered strains that reproducibly form cancers (b) use new imaging methods to follow cancer growth and treatment so that small numbers of animals can be studied (c) derive cancer cells and develop new methods for their analysis to further reduce the numbers of animals used, and (d) use based on statistics the minimum numbers required for each experiment.
3. 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 laboratory mouse is recognized to develop cancers that are similar enough to their human counterparts to provide important information on human cancer formation, detection and treatment. This species is (a) readily amenable to genetic alteration, which enables creation of strains that reproducibly form cancers similar to their human counterparts, and (b) by far the most studied in cancer research, providing considerable background knowledge to reduce usage and refine experimental procedures. Through past research, we have

	developed strains carrying mutations affecting human cancer genes, which reproducibly develop cancers that closely resemble their counterparts in humans, making this the most refined model for our studies. To minimise harm to the animals, we will (a) use the minimum numbers required (b) refine our experiments to reduce suffering, for example using imaging methods (c) monitor animals regularly for signs of harm by physical examination or other tests, and (d) take immediate steps for humane treatment should signs of harm be found. We will house mice in groups where possible, enrich their caging environment, and provide suitable bedding.

Project 22	**Genetic analysis of tumour development**	
Key Words (max. 5 words)	Cancer, tumour, inflammation, therapy, imaging	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Cancer can start when a group of cells in the body gain the ability to divide in an uncontrolled manner, ultimately leading to the formation of a tumour (lump). However, it has recently been recognised that for tumours to grow and spread, they must develop a supportive environment, primarily through re-educating certain cells of the immune system, to develop blood supply and protection against toxic agents. Thus, a therapeutic strategy that attacks cancer cells, whilst concurrently neutralising immune cells, has become a very attractive option by which to advance cancer treatment. This approach is supported by encouraging results in clinical trials where blockers of certain immune molecules have stabilised the disease of patients with advanced cancer. However, the benefit of blocking a single immune molecule may be brief because tumours produce multiple immune molecules. Therefore, we need to improve our understanding of how the different cell types in the tumour interact to develop more effective cancer treatments.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	The primary potential benefit relates to new knowledge about tumour initiation, development and resistance to therapies. The aim is to disseminate our findings through academic publications and oral presentations. This information is of interest to	

project)?	biological scientists. The secondary potential benefit relates to clinicians, in particular oncologists. New molecular targets may be identified, for which pharmaceutical products could be designed. Imaging modalities may be developed to facilitate early clinical evaluation. A reduction in invasive procedures to assess tumour biology in pre-clinical models of cancer is anticipated.
What species and approximate numbers of animals do you expect to use over what period of time?	6000 mice
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?	This project will create, breed and maintain mice with appropriate genetic changes. For the majority of animals, we anticipate that these genetic alterations will have negligible adverse effects and be of mild severity. Some genetic modifications may result in abnormalities causing moderately severe suffering. These will be minimised by appropriate breeding and husbandry methods. Tumours will be induced by expression of disease relevant genes, repeated exposures to chemical carcinogens, or injection of tumour cells. These models are chosen based on comparable disease aetiology with humans. The overall health of mice bearing benign tumours is generally acceptable, unless their number or size becomes excessive leading to distress. Consequently, tumour burden that will not exceed 5% of the host animal's normal body weight should avoid undue discomfort. Animals displaying invasive and internal tumours will be monitored regularly to allow detection of progression of malignancy. In the majority of instances, termination by a humane method at defined ages will be required. Substances may be administered in the diet or drinking water, by direct application onto the skin or by experienced licensees via oral gavage, enteral or parenteral routes. These procedures should not result in more than temporary pain, suffering or distress. If required, some procedures will be performed under light general anaesthesia and/or pain relief. Mice will also be used to develop imaging strategies to evaluate tumour development and changes in tumour biology caused by genetic modification and/or administration of a therapy, which include

	radiotherapy and targeted agents.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The plan is to identify new molecules underpinning the communication between tumour cells and their environment. This will involve scientific experiments using live mice, as it is not feasible to produce an adequate in vitro model that satisfactorily replicates the complex interactions between the different cells in the tumour.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of mice required for each experiment has been calculated based on our extensive experience working with mice and with the advice from statisticians. Under most situations, the demonstration of similar effects in distinct animals (3 to 6) of identical phenotype is sufficient to establish the effect and rule out artefacts associated with biological variability. These experiments will be statistically designed to get as much information as possible with the least number of animals to be sacrificed.
3. 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 constitutes an appropriate species because fundamental biological and pathological processes are similar or identical to those in other mammals, including humans. The mouse genome has been sequenced and can be manipulated to create genetically modified models. These models can be subsequently used to understand the function of genes. Animal suffering will be minimised by daily monitoring and by ensuring that staff are fully trained and assessed as competent. Where any signs of distress are present in an animal, then the animal will be euthanized immediately and any remaining mice of similar genotype observed closely for changes in their condition.

Project 23	**Tumour models for therapy of advanced cancers**	
Key Words (max. 5 words)	tumour, metastasis, novel drugs, imaging	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to utilise clinically-relevant animal models to understand the role of specific genetic abnormalities causing cancer development and progression and to evaluate novel therapeutic approaches for advanced malignant disease.	
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)?	Once cancer spreads (metastasises), cure rates significantly diminish and over 90% of cancer deaths are due to secondary cancers at distant sites in the body. We are developing therapies targeting genetic alterations associated with tumour growth, tissue invasion, cancer spread and also the cancer's blood supply (angiogenesis) on which sustained growth and the opportunity to disseminate via the blood stream depends. We need to model both common cancers and rarer cancers which are hard to cure and also their major sites of metastasis to ensure that our new drugs are capable of tackling these unmet clinical needs. While targeted therapies have shown some promise, the development of drug resistance and the need for rationally-designed combinations of agents is becoming a major issue which will be addressed in this licence. We measure the efficacy of an agent (on primary tumours and/or metastases) in relation to its levels in the blood and/or tumour to inform the optimum starting	

	dose and schedule in man. In parallel, we develop quantitative biomarkers of response which help us to understand determinants of sensitivity or resistance and to confirm that efficacy is tightly linked to the desired mechanism of action. This knowledge and the technology we develop in our models is directly transferrable to the clinic. We also check normal tissues at autopsy, and aim to define the 'biologically effective dose': the minimum dose of the drug that gives therapeutic benefit without significant adverse effects. The most promising compounds proceed to clinical development and trials in cancer patients.
What species and approximate numbers of animals do you expect to use over what period of time?	We use immunocompromised mice -the simplest species suitable for such complex pathophysiological studies in which human tumour cells can be grown. Over a 5-year period, we expect to use no more than 7000 mice
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?	All procedures are designated as moderate. Adverse effects are related to the implantation and growth of tumours (superficial and within internal organs) surgery to remove primary tumours, ovaries or testes, anaesthetics for surgery or imaging and the effects of therapeutic agents. All animals will be humanely culled by a Schedule 1 method or by collecting blood at the end of the studies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Human cancers develop in 3-dimensional (3-D) space within specific tissues in the body, each providing a unique growth environment which cannot be adequately modelled in 2-D cell cultures grown on plastic dishes in the lab. Cultured cells are provided with constant, optimal levels of oxygen and nutrients, and are all growing at the same rate. This is rarely the case in the body, and variations in these parameters can significantly influence responses to therapy. Metastasis in particular (the major cause of treatment failure) is exclusively an in vivo phenomenon, since during this process tumour cells from a primary cancer must access the blood circulation, spread around the body and colonise new organ sites. Similarly, the effects of drugs must be tested in vivo to determine that adequate levels are achieved in tumour tissues, that adverse effects on normal tissues are minimised and that efficacy tracks with effects on appropriate biomarkers.

2. Reduction Explain how you will assure the use of minimum numbers of animals	All compounds are first tested in tissue culture for potency, specificity and stability using both simple tumour cell monolayers and more complex 3-D functional assays (e.g invasion). Failure at any of these stages, limits the number of compounds going forward for testing in animals. As far as possible we use cells in which we express luminescent or fluorescent markers that emit light, enabling detection of tumours inside mice using optical imaging, which is quick and requires only light anaesthetic. Otherwise we use methods such as magnetic resonance imaging or ultrasound to locate and follow the development of internal tumours and their response to therapy. Thus fewer mice are required and studies can be terminated before the animals experience significant symptoms. We ensure that we obtain the maximum possible information from each tumour, assessing not only tumour growth rates but also correlating efficacy with drug levels and biomarker responses to give statistically robust data in proof of concept tria!s.
3. 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.	Mice are the lowest species that are appropriate for in vivo drug development studies and are widely used for this purpose. Most of our work is carried out using well-characterised human tumour cells, grown in the appropriate anatomical site in naturally immunodeficient adult mice to avoid tissue rejection. This enables us to study human cancers in the correct tissue microenvironment, The animals are maintained in individually ventilated cages using sterile food and bedding and all procedures are carried out in special cabinets using strict aseptic techniques to avoid infections. Suffering will be minimised by keeping tumour burdens within tolerable and acceptable limits and according to NCRI guidelines. Compounds to be evaluated will have been selected for potency, stability and tolerability in other projects. They are delivered using previously determined well- tolerated doses and schedules, and are generally of low toxicity (e.g. agents targeted to molecules selectively overexpressed or mutated in human cancers).

Project 24	**Understanding inflammation, fibrosis and cancer**
Key Words	Scarring, cancer, drugs
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;

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

Fibrosis is characterised as the build up of scar tissue in a damaged organ and its gradual accumulation correlates with a steady decline in organ function.

Fibrosis can affect many organs and some of the events controlling the development of fibrosis that are common in different organs regardless of the type of injury to the organ. It is thought that fibrotic disease follows a common course; repeated organ damage → persistent inflammation → fibrosis → increased risk of developing cancer. This is known as the “inflammation-fibrosis-cancer axis”. Additionally the cells that make scar tissue in different organs are similar; therefore a drug that prevents fibrosis in the liver could also prevent fibrosis in the lung, skin or heart.

Currently there are no drug treatments for fibrosis in any organ and the only therapy for liver cancer only extends life by 3 months.

OVERARCHING AIM: Use a combination of physiological, biochemical, histological and molecular approaches to identify proteins and signalling pathways that influence every point of the inflammation-fibrosis-cancer axis. Our research plan is to use a multi-organ approach to understand the “core” molecular events underpinning these diseases to discover new targets and test new drugs to treat fibrosis.

Objective1: understand how multi-organ fibrosis develops and identify potential drug targets and therapies to prevent these diseases.

Objective2: understand how liver cancer develops.

Scientific Aims/Key elements:

1. To understand the biology of wound healing and fibrosis in the liver, skin, knee/joint, heart or lung.

2. Understand the molecular and cellular mechanisms promoting liver cancer.

3. Understand how epigenetic changes that occur as a consequence of CLD/fibrosis can be transmitted to future generations to protect offspring from developing CLD and fibrosis.

4.Test therapeutic agents that target signalling pathways or molecules, or modify lifestyle (e.g. diet and/or exercise) contributing to the inflammation-fibrosis-cancer axis.

5.Develop a rodent model of arthrofibrosis and test potential therapeutic compounds.

6.As part of the overall program of work, to develop and refine imaging techniques to monitor fibrosis and liver cancer.

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)?

The primary outcomes of our work are; 1. To identify new drugs that prevents fibrosis in different organs. 2. Identify biomarkers or imaging tools to help understand/study the progression of fibrosis or tell us that fibrosis is reversing e.g. if a drug is working. Biomarkers include molecules or proteins released into the blood or changes to the epigenetic code in liver tissue. 3. Develop new imaging methods to detect/visualise fibrosis in organs. Clinical Importance: Organ fibrosis is a debilitating disease and as scars accumulate the ability of the organ to do its job declines. It's estimated that fibrosis may cause up to 45% of deaths in the western world. Treatments for fibrotic diseases are limited because there is a lack of effective drugs. The number of people with fibrosis is rapidly increasing; therefore managing this disease epidemic is creating significant social, economic and healthcare burdens. Our programme of work will help us understand how inflammation (redness and swelling caused by white blood cells) and fibrosis (scarring) occurs in an organ to identify and test new medicines to treat these diseases. We want to understand how liver cancer develops and test new drugs, which prevent cancer growth. We also hope to develop new imaging methods to help assess liver disease in living animals.

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

Adult mice and rats. We estimate that over 5 years we will use up to 30,000 mice for maintenance and breeding of different mouse lines. Experimental procedures are estimated to be up to 28,900 mice and up to 600 rats over 5 years. The animals will be either generated in-house or purchased from an authorised supplier.

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?

All procedures are classified as moderate except acute carbon tetrachloride (chemical) liver injury (mild), cell isolation from liver tissue (non-recovery), and protocols surgically tying off the bile duct & liver failure models e.g. paracetamol overdose (severe). At the end of all studies blood samples and organs will be taken to analyse the extent of disease and determine if therapies have been successful. Mice will be given chemicals (carbon tetrachloride or thioacetamide) or surgically injured (tying off the bile duct) or fed a western lifestyle diet e.g. high fat, high sugar diets to cause scarring in the liver. Adverse effects: Mice will develop liver disease and very rarely show signs of sickness e.g. hunched posture. Bile duct ligated (BDL) mice, where we surgically restrict the bile duct, occasionally become jaundice (skin becomes a yellow colour) or get swelling of the belly.

In the dietary models most mice will gain weight, but in the methionine and choline deficient (MCD) diet model we expect mice to loose weight. Liver growth is assessed by surgically removing up to 70% of the liver. There are no adverse effects in the liver growth model other than potential surgical complications; bleeding or inadequate anaesthesia. Liver cancer: we give mice a chemical that causes liver cancer to develop over a period of up to 60 weeks. Adverse effects: Mice will develop liver cancer but this will not result in liver failure. Liver cell death will be promoted by giving mice chemicals or drugs at toxic doses (e.g. paracetamol). Adverse effects: The liver injury caused by the drug/compound will be either lethal where mice are humanely killed as the liver fails or sub-lethal where mice will recover.

Lung inflammation/scarring will be induced by putting chemicals or allergens in to the lung to cause damage. Adverse effects: Mice will develop lung disease and can loose weight during the first week of the models but regain weight after this time. Skin inflammation/scarring will be induced in the mice by either giving agents, which irritate the skin or chemicals that cause scarring. Punching two small holes in the skin and then watching them heal will be used to study how skin wounds heal. Adverse effects: The skin may become red (inflammation) and the skin will become thicker. The wounds created by “skin punching” are superficial and therefore do not bleed.

Cardiac fibrosis will be induced by increasing blood pressure, which stresses the heart by chemical (angiotensin) infusion or surgically restricting the aorta. We may directly injure the heart by freezing a small area of the heart; once damaged cells are lost they are replaced with scar tissue. Adverse effects: The heart will become fibrotic, but the extent of the injury is not sufficient to cause a heart problem. Knee fibrosis will be induced by surgically removing/partially removing the fat pad in the knee joint. Adverse effects: The knee may be stiff and the joint movement slightly restricted.

Bone marrow chimera: normal or genetically modified (GM) mice will be irradiated to remove the white blood cells and then given a new immune cells from a donor mouse of a different background e.g. normal in to GM. Adverse effects: Mice may be sick and loose weight after irradiation but will recover once the immune system has been replaced. At the end of the experiment animals will be humanely killed, the affected organ (liver, lung, skin, heart or knee) will be removed and blood samples will be taken to assess the extent of disease. We will use microscopes to look at scar

cells, white blood cells, dividing or dying cells and scar production. We will measure damage markers in the blood.

Application of the 3Rs

Replacement

Organ fibrosis or development of liver cancer is a complex process. These diseases develop over many weeks and require different cells both within the organ and within the blood to talk to each other.

In our “inheritance” study where we are asking how protection from developing fibrosis can be passed from father to son. To achieve this we need to promote fibrosis in the dad, then breed him with a female mouse and then induce liver fibrosis in their offspring and then compare the amount of fibrosis to mice breed from fibrotic or healthy parents.

Therefore these programmes of work can only be conducted in animals and not alternative systems such as growing cells or organ slices in a petri dish. Wherever possible, we use human tissue to isolate cells or make slices, which we can culture in a petri dish to replace animal models of fibrotic disease. We use stored frozen and wax embedded liver tissue collected from previous studies to help answer our research questions and minimize further use of animals.

Reduction

Studies are planned very carefully. We always use the minimum numbers of animals possible to achieve meaningful results. Statistical analysis is used to help predict the number of animals needed to achieve our research aims. We use imaging approaches to help reduce group sizes or numbers of time points which reduces numbers of groups.

Refinement

All of the disease models described in the plan of work are the least severe models that will allow us to answer our research questions and achieve our aim of further understanding how inflammation, scaring (fibrosis) develops in multiple organs. This will help us identify new drug targets and test potential drugs, which inhibit these targets to develop new anti-inflammatory and anti-fibrotic medicine.

We have good surgical techniques and mice or rats used in surgical models will receive pain relief and a high level of post-operative care to minimize stress and suffering.

We will use methods to watch the mice and perform behavioural tests to help refine our procedures. We will monitor all of the animals carefully, and apply well-defined humane endpoints to limit the severity of the disease processes. We will provide supportive care (for example fluids and more palatable food) to reduce the effects of the disease process.

All models will be performed in mice except surgically inducing fibrosis in the knee, which will be performed in rats. This is because disease processes are complex and involve lots of different cell types, therefore to understand the disease process we

need to use whole animals. Mice are used because the disease models have been very well characterised in this species. In addition, we can genetically modify mice to help understand the role of specific factors involved in the disease process. Rats are use for the knee fibrosis because their joints are larger which is more amenable for surgery and allows the disease to be assessed more accurately and offers a better system to test new treatments.

Project 25	**Signalling pathways in cancer, inflammation, and metabolism**
Key Words	NF-κB, cancer, therapy, inflammation, metabolism
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;
Yes	(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);

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

NF-κB denotes a family of proteins which govern the body defence responses to injury, infection and stress. NF-κB however has also been linked to cancer, inflammatory and autoimmune diseases, septic shock, viral infection, and improper immune development. The aim of our work is to gain a better understanding of how NF-kB promotes cell survival, tumour development, and inflammatory and metabolic diseases, and develop safer and better treatments selectively targeting NF-kB in patients suffering from these illnesses.

Aim 1: Investigate the role of NF-kB in tumour development, and gain a better understanding of the molecular mechanisms by which NF-kB promotes this process. We will investigate the involvement of NF-kB in both solid and blood cancers, and delineate how NF-kB operates in these contexts.

Aim 2: Develop new therapeutic strategies to selectively block NF-kB in cancer, and inflammatory and metabolic diseases, and promote the development of new and more specific therapies, which are safer and more effective than the current non-specific treatments aimed at blocking NF-kB.

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)?

Currently there is an urgent medical need for developing improved therapies to treat various types of blood and solid cancers, as well as inflammatory and metabolic

diseases. The work proposed under this License application will deliver these objectives in various areas of unmet medical need, both within and outside of oncology, including solid and blood cancers, and inflammatory and metabolic diseases.

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

Only mice (both wild type and genetically modified) will be used for this project. This is a 5 year project License, and we estimate that for all the procedures outlined in this License up to approximately 7,330 mice per year will be used.

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?

None of the experiments planned under this project License involve procedures that are expected to cause the mice severe distress or discomfort. All procedures have been designed to detect any animals that appear to be suffering and killed them, if required, by using humane methods. Genetically modified animals, including animals that display a genetic predisposition to tumour development, will be used and bred under this License. For this reason, mice will be carefully monitored for possible side effects and specific endpoints have been adopted to minimise the suffering of the animals and limit this to what is strictly necessary for the research. A small proportion of the animals used in this Licence will undergo bone marrow reconstitution, following irradiation. To avoid infections and suffering to them, these animals will be inspected daily and several refinement measures have been adopted in order to ensure the animal wellbeing. Drugs will be administrated to a small proportion of the mice used in this License in order to study their therapeutic effects on tumour growth. Additional humane endpoints have been designed in order to avoid any suffering to these animals. A very small proportion of animals will undergo a moderate surgical procedure (e.g. the implantation of small drug delivery devices under their skin). To minimize any associated suffering to these mice, we have taken the veterinary advice, will utilize anesthesia and analgesia to control any pain, and will terminate the experiments early, if required, should the animals show any sign of discomfort or distress. Some of the animals used in this License will be exposed to tumour challenge, whereby the tumour may arise either spontaneously or after the injection of tumour cells. This is necessary to help us to understand how tumours develop and grow, and to find better treatments for patients with cancer. Occasionally, we will need to monitor tumour growth in these mice by taking blood samples or using imaging techniques similar to those used in the clinical practice. For mice undergoing each of these procedures, additional humane endpoints have been adopted in order to avoid any unnecessary suffering to them. In the case of development of unusual or unexpected clinical signs or adverse effects, we will seek advice from the Named Animal Care and Welfare Officer (NACWO) and/or the Named Veterinary Surgeon (NVS) for taking the appropriate measures.

Application of the 3Rs

Replacement

While valuable studies of human cancer are performed using human 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.

Use of refined animal models is thus the only valid way of both advancing basic understanding of cancer development and evaluating novel approaches to treat these diseases.

A search of the Altweb web site(http://altweb.jhsph.edu/resources/searchalt/searchaltdata.html) and a subsequent search of the NIH web site (http://emice.nci.nih.gov/aam/mouse/how-and-why-mouse-cancer-models-are-used) have confirmed that, on the basis of the aforementioned considerations, there are no suitable alternatives to mouse models in research aimed at delineating the processes governing inflammation, immunity and oncogenesis, as required by this project.

Reduction

The following measures will be routinely adopted for reducing the number of mice:

1. Mouse colonies will be closely monitored to avoid excess animals. As appropriate, we will plan effective mouse breeding strategies which can provide us with the required experimental mice and controls.
2. In the majority of experiments, post mortem tissue will be harvested at the end of experiments to gain invaluable *in vitro* immunological and molecular analyses.
3. *In vivo* experiments are carefully designed to use the minimum number of animals, whilst providing meaningful and statistically valid outcomes.
4. Only certain strains of mice, which reduce the biological variability due to genetic factors, will be used.
5. Comparison will be made only between strain-, sex- and age-matched groups, and equal number of mice will be used in each group to avoid statistical artefacts.

Experimental design:

For those experiments where outcomes in experimental and control groups of animals are compared, it is important that group size is sufficient to demonstrate whether there is a statistically significant difference, whilst keeping the numbers of mice as low as possible (https://www.nc3rs.org.uk/experimental-designstatistics). In all cases, we will aim to minimise animal numbers consistent with achieving scientifically and statistically robust results.

Refinement

Appropriate guidelines for good practice will be followed. Animals will be inspected regularly to ensure general wellbeing and any animal showing signs of adverse

effects will be humanely killed by a Schedule 1 method. The NVS and/or NACWO will be consulted for advice where appropriate.

We will follow the following guidelines:

- Lasa Good Practice Guidelines on ‘Collection of Blood’ and ‘Administration of Substances’ will be followed.
- The NCRI guidelines for the Welfare and Use of Animals in Cancer Research for endpoints in experiments involving tumour development will be followed.

To refine the experimental conditions and to ensure minimal suffering, the following steps have been taken:

1. Where possible, a short-acting general anesthetic will be used
2. Pre- and post-operative analgesia will be routinely administered
3. Where possible, vasectomized males will be replaced by genetically sterile males
4. Non-invasive imaging may be used for direct assessment of tumour growth in systemic tumour models. This is a refinement over indirect (*e.g.* loss of condition) indications of tumour growth

Bioluminescence and ultrasound imaging, two non-invasive methods which increases the quality and quantity of the experimental data obtained from a single experiment, will also be used on animals under general anaesthesia. This will dramatically increase the information that can be accrued from each mouse.

Project 26	**Platelets and cardioprotection in cancer**
Key Words	Platelets, cancer, cardio-toxicity, metastasis
Expected duration of the project	5 year(s) 0 months

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

Purpose

(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;

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

We aim to investigate the role of platelets, a component of blood, on the spread of cancer and to study the potential of drugs targeting platelets as future treatments. We also aim to study whether treatments can be found to protect against heart injury caused by cancer treatments.

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)?

We hope this research will lead to new treatments for cancer which reduce spread by impacting the survival of cancer cells in the blood and therefore reduce the likelihood and rate of cancer spread. We also hope this research may lead to treatments which protect against heart injury caused by chemotherapy and potential enable higher doses of cancer treatments to be safely used resulting in better outcomes for patients.

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

We propose to use up to 10500 mice and 100 rats over 5 years

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?

Several types of animal models will be used: Some animals will be either given treatment or genetically modified to alter the function of platelets or other components of blood which interact with cancer cells. Other animals will be given anticancer treatments which cause heart injury. Other animals will be given treatment or genetically modified to develop cancer. These models may also be used in combination. This will be necessary to allow the research team to investigate the effects of the treatments under investigation and to understand how and why they

work. Animals will be carefully monitored throughout the experiments to ensure any symptoms are minimised. At the end of the experiments animals will be humanely destroyed.

Application of the 3Rs

Replacement

The research group are also studying aspects of these research questions in cells and by using human blood. However it is essential to understand the effects of the treatments under investigation in a whole animal before experiments can advance further in people as cells cannot mimic the complexity of the process of cancer metastasis or cardiotoxicity.

Reduction

We will use cell studies and studies using human blood where possible We will carefully design our experiments to use the minimal numbers required to demonstrate whether or not a potentially important effect can be identified.

Refinement

We will use mice and rats for these studies as there is considerable experience of these species for the study of cancer metastasis, platelet biology and cardiotoxicity and these animals are a good model for human disease. Animal suffering will be minimised by careful observation and scoring of signs of illness, pain or distress to ensure animal welfare is maintained.

Project 27	**ONCOLOGY MODELS**
Key Words	Oncology, Immunology, Therapeutics
Expected duration of the project	5 year(s) 0 months

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

Purpose

(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;

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

There are a large number of potential causes of cancer in humans, including genetics, diet and lifestyle. While some cancer types have successful treatments, there are others that require new therapies because current treatments can only slow rather than cure the disease.

The primary aim of this programme of work is to progress novel therapies for these human diseases through addressing this unmet need. Novel therapies will be tested in animal models of human tumours to assess how effective these may be in treating human cancer.

Before animal testing, any potential new therapies will be tested in the lab to ensure that only the most promising candidates will be progressed into animal studies. There are currently no lab-based approaches that are sophisticated enough to replace animal studies of human cancers; these studies are of vital importance to inform us of which of these potential treatments will have the greater likelihood of working in human disease.

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)?

The overall benefit of the work on this licence is to develop improved therapies for patients with these diseases. Cancer is a major group of diseases that affect a large proportion of the human population worldwide, with approximately 14 million new cases seen each year. Cancers as a group account for approximately 8 million deaths per year, roughly 13% of all deaths each year. While there are many cancers which are curable, there are currently cancer types for which there are treatments that only slow the disease or reduce symptoms in some patients. Results from studies performed under this licence will provide key information on the likelihood of a particular new therapeutic working in these types of diseases.

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

We expect that up to 5,500 mice will be used over the 5 year licence duration.

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?

The mice used under this licence are generally likely to have deficient immune systems. This allows them to act as hosts to human cancer cells, as their immune systems are not strong enough to reject the human cells. We need to study the effects of novel therapies, not only on the cancer itself but also on the whole animal. Some animals will have their immune system modified and the effects of this will be studied. Animals may develop cancerous lumps after cell implantation; though adverse effects from these implantations should be limited, effects such as weight loss in some animals may be seen. Some animals may require surgery to implant slow-release tablets or tiny pumps to provide essential growth factors for tumour cell growth, or to administer therapies. Anaesthetics and analgesics will be used to reduce the discomfort induced by any surgical procedure. We will administer novel therapeutics, take blood samples, measure markers of the treatment response and determine whether these therapies can affect the disease. Some animals will experience discomfort due to tumour growth or from dosing of potential new therapeutics. Any animal displaying discomfort and distress beyond the minimum required to achieve the aims of the study (e.g. pilo-erection, hunched posture, subdued responsiveness, and weight loss of no more than 20%) will be euthanased humanely. Should any animal not be able to display normal behaviours, eating, drinking, grooming, walking, rearing, etc. associated with its species it will be euthanased if normal behaviour cannot be restored quickly by veterinary treatment and/or care. At the end of studies the animals will be euthanased and samples taken after death to maximise the scientific information we can gain from the animals.

Application of the 3Rs

Replacement

Prior to any animals being used on this licence, the potential new therapies will be tested in the laboratory to ensure that only those most likely to become new therapies will be progressed into animal studies.

The clinical progression of disease, such as cancer, involves numerous systems within the human body which play key roles and replicating this scenario in animals is critical to developing new therapies.

In addition complex interactions involving patterns of tumour growth, establishment of a blood supply and interactions with the immune system cannot be replicated or understood without the use of animals.

Reduction

The estimated number of animals is based on our previous experience of successfully designing these types of studies to develop new therapies minimising

the numbers of animals used whilst ensuring meaningful results. For new study designs we will consult with a statistician to ensure that we are using the minimum number of animals to achieve the licence objectives.

Refinement

The species used on this licence will be mice, most of which will have deficient immune systems to allow human cancers to grow. The most appropriate strains will be selected for each animal study, which exhibit the desired traits that best mimics the disease in humans. This gives a greater chance of the treatment working in human disease.

The volumes of injections and the number of blood samples taken will be the minimum to ensure that the welfare of the animal is not compromised and that the level of discomfort is kept to a minimum, while achieving the scientific aims for these studies. Surgical procedures will only be performed using appropriate analgesics and anaesthetics to minimise discomfort. Refined technical procedures will be used to minimise the impact on the individual animal.

Animals will be routinely group housed with appropriate litter, nesting material and environmental enrichment.

Veterinarians will be available to give advice on care for the animals and can be contacted outside normal working hours if necessary by scientists and animal care staff.

Project 28	**Haematopoietic and leukaemia stem cell regulation**
Key Words	Haematopoiesis; Stem cells; Leukaemia; Lymphoma; Leukaemia stem cells; Novel Therapy
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;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(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);

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

The key questions and objectives are:

1. Identification of the molecular and cellular events involved in the formation, maintenance, expansion and differentiation of HSPC and characterisation of how these regulatory events differ between normal and leukaemic stem cells?**.**
2. Can we generate appropriate mouse models to study critical genes in HSPC and haematological malignancies to improve our understanding of their pathogenesis?
3. Can we validate critical regulators of leukaemia and lymphoma biology and obtain pre-clinical information on novel therapeutics targeting these regulators?

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)?

This programme aims to identify molecular and cellular regulators of stem and progenitor cell fate choice. Manipulation of this balance is suspected to play a major

role in disease development. This programme also aims to develop relevant models to understand human disease and provide a system where new therapeutic strategies and compounds could be tested for their ability to reduce disease burden in patients. We anticipate identifying 3-5 critical regulators of haematopoietic stem cell function and blood formation. Characterisation of each model's relevance to disease requires careful and time-consuming experimentation but it is anticipated that one or two of the models created in this programme will be used to test potential therapeutic strategies for haematological malignancies.

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

Species: Mouse: We expect to use approximately 6,768 animals per annum over 5 years. (i.e. ~ 33,820 animals during this project.)

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?

Since this project will explore the role of previously unstudied genes in the blood system, it is likely that disease will develop in some of these animals, including leukaemia or lymphoma development. Animals will be very closely monitored for signs of disease and killed by a schedule 1 method should the clinical signs necessitate intervention. The majority of mice under this licence will show no signs of adverse effects that impact materially on their general health. It is estimated that more than 40% of animals will not exceed a sub-threshold severity category. Approximately, a further 20% may not exceed mild and ~25% may reach a moderate severity category. Rapid and/or unanticipated adverse effects may rarely be such that the some animals (less than 5%) reach a severe category. Over the last 2 years this has been between 2.8 and 3.2%. These animals may develop haematopoietic malignancies causing abdominal distension, weight gain or loss, anaemia or erythrocytosis, laboured respiration, inactivity or inappetence, combined with signs of hunched posture or piloerection. Animals showing any of these clinical signs will be deemed to have reached human end points and will be immediately killed by a schedule 1 method.

Application of the 3Rs

Replacement

It is currently impossible to study the complex role of the microenvironment of normal and malignant stem and progenitor cells *in vitro* since the complete set of important factors have not yet been identified. Also, to study stem cell function, the cell must be shown to possess the ability to sustain lifelong blood cell production and this cannot currently be assayed outside the body. Finally, to assess the function of normal and patient derived human stem and progenitor cells, the xenograft model (where human blood cells are transplanted into mice with compromised immune systems to avoid rejection) is currently the only system capable of determining long-term multi-lineage capabilities.

Reduction

Colony sizes will be carefully managed to ensure that supply matches the demand, and any surplus mice are used for other scientific purposes and tissues shared over multiple experiments. When designing experiments we perform statistical analysis (e.g., power calculations) to ensure that we use the minimum number of mice per group that will be informative. Finally, we will use human cell lines (including patient derived cell lines) of particular mutations (e.g., *CREBBP, DNMT3A*) to study the biochemistry of the mutations.

Refinement

The mouse is the most appropriate and most widely used model for studying blood cells and cancer. The techniques are therefore very well established and findings can easily be integrated with other groups' data. The mouse is also the species in which reliable gene delivery systems are best established. For our studies that involve blood cell transplantation, we have recently introduced a recipient mouse model that permits much lower irradiation doses and we have also removed techniques that are no longer required from our licence.

Project 29	**Novel strategies to target cancer**
Key Words	Cancer, Microenvironment, Immunotherapy
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;

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

Numerous scientific observations suggest that how a particular cancer develops, progresses, and responds to therapeutic interventions is dependant not just on the cancer cells themselves, but also on the interactions of the cancer cells with the non-cancerous cells in the host. These interactions take place not just in the immediate vicinity of the cancer, but also more widely throughout the body. An example of the latter is the positive effect observed from exercise programs on long-term cancer outcomes.

Nevertheless, our current understanding of the mechanisms underpinning these key observations is limited, and clinically useful translation of this important field is under-exploited.

As a result, the proposed program of work seeks to identify and investigate novel and fundamental tumour-host interactions that can influence cancer progression and its response to therapy.

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)?

• Acquisition of new and fundamental biological understanding • Better understanding of human cancer and identification of novel methods of treating it. • Better understanding of veterinary cancers and identification of novel methods of treating them. • Immunotherapy and harnessing the host system better to combat cancer offers the potential for treating a wide range of cancers with highly specific ‘medicines’, in the same way that patients currently receive vaccinations to protect against infectious diseases. Moreover, these “living drugs” offer the tantalising possibility of successful treatment of a cancer forever, leading to a permanent cure.

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

We are investigating complex interactions between cancer and different tissues/organs to improve human cancer treatment; such interactions cannot yet be reproduced in the laboratory, necessitating animal use. However, technological advances continually permit experimental refinement, replacement and reduction of animal numbers (the 3R's); and this is something to which we remain committed. Mice are the least sentient mammal species with an immune system similar to that of humans. Numerous mouse cancers have been studied and the availability of genetically altered strains aids this research. Per year, approximately 1000 mice will be used, plus small numbers of rats (~30) (e.g. for tissue samples). Experiments are designed to involve the lowest number of animals consistent with obtaining statistically valid results. Where appropriate, small pilot experiments will check outcome and assess unexpected, harmful effects before further experimentation. Environmental enrichment, good husbandry and frequent monitoring will ensure high welfare standards. Few adverse effects are anticipated but, should any occur, rapid steps will be taken to ameliorate them or humanely cull affected animals. Death is not an acceptable end-point for cancer models: those that we use will have an established end-point for humane culling of affected animals before pain/distress occurs. End-points are based on accepted guidelines and pilot experiments will establish these for new cancer models.

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?

Expected adverse effects are the development of cancers in animals, and the potential side-effects of multi-modality treatments on them including surgery, chemotherapy, and or radiotherapy, aiming to recapitulate contemporary human cancer therapy as faithfully as possible. Expected level of severity is moderate. All animals will be culled at the end of the research.

Application of the 3Rs

Replacement

This project aims to identify and investigate new and fundamental interactions between cancer cells, and their surrounding (or even more distant) non-cancerous cells, and how these contribute to cancer spreading from one anatomical site in the body to another. Subsequently, we hope to use the results uncovered to develop improved methods of treating human cancer.

Although we and other groups have developed and published on complex synthetic 3-dimensional model systems for the study of cancer cells, unfortunately these fail to fully recapitulate the real-life complex and multi-cellular findings in a typical cancer, and hence our ability to learn from them is ultimately limited. Even more importantly, they do not replicate the spread of cancer from one site to another. In light of the fact that this spread is in fact responsible for the vast majority of cancer deaths, the study of this field is exceptionally important.

In addition, the testing of combinations of conventional drugs, radiotherapy, and surgery as takes place in man, or more modern agents derived from our ongoing research studies, again cannot be properly performed or interpreted without the use of animal models that provide the complex living physiological system for study.

Nevertheless, this study is well supported by an extensive array of non-animal models, and we will use these whenever possible.

Reduction

A key factor in experimental design will be reduction in animal numbers.

Most experiments in this proposal will use inbred strains of mice, to reduce experimental variability, and to therefore allow completion of objectives with the smallest numbers possible.

In all cases, numbers used in any experiment will be based on initial in-house or external collaborator conducted pilot studies with subsequent statistical calculations to determine the absolute minimum number of animals required in each experimental group to observe an effect.

Additional attempts to reduce numbers are conducted by using test-tube and cell model systems for tests rather than animals wherever feasible.

Refinement

This project is principally concerned with the efficacy of therapeutic strategies for cancer treatment, gained from the study of the interactions between cancer and its environment, rather than pharmacological or toxicity research. We therefore select agents/delivery techniques, and tumour models from published/commercial/research sources. Consequently, adverse effects are usually known or available knowledge will be applied to predict them. Should substances be used for the first time in mice (unlikely), individual mice will be treated and monitored closely for adverse effects before proceeding; and a dose escalation schedule conforming to accepted practice will be used should adverse effects be predicted.

Species

Mice will be used for most of our research. They are the least sentient mammal commonly used in research with an immune and non-immune micro/macroenvironment similar to that of humans. Numerous mouse tumour models and strains of genetically altered mice are available, have been published on, and help facilitate this research. Occasionally, we may use small numbers of rats for tissue samples or to test model efficacy should mice be unresponsive; this species has the next lowest neurophysiological sensitivity likely to produce satisfactory results. Cages always include environmental enrichment to satisfy normal species-typical behaviour.

Tumour models

Animal death is not considered an acceptable endpoint measure. Our humane endpoints are based on recognised guidelines (e.g. Workman et al. [2010] Br J Cancer 102:1555). Cages housing tumour-bearing animals near the critical end-

stage of disease are marked for special attention. Daily inspections are usually carried out by experienced animal technicians familiar with the models. If an animal has reached its humane end-point or is displaying signs of distress/suffering, it will be humanely culled. . Pilot experiments will be undertaken to establish humane endpoints for unfamiliar tumour cell lines. An end-point index will be maintained in-house.

Many tumour lines can develop as subcutaneous nodules, allowing easy monitoring. However, certain tumours develop internally, homing to specific organs and/or diffuse disease; or as a result of orthotopic implantation. In these circumstances, assessment of tumour development can be more difficult, however our experience with in vivo live animal imaging using a recently acquired IVIS Lumina III imager (Perkin Elmer) has allowed us to evaluate size more robustly in these circumstances. In addition, through careful monitoring of established end-points, the proportion of unculled deaths is low, ensuring that moderate severity is not exceeded.

Surgery will only ever be conducted by suitably trained senior surgical trainees (most common situation) or consultants, or laboratory staff with a high level of skill and dedicated training in the procedures and under the supervision of clinicians accustomed to human and or animal surgery. Our current experience of surgery was developed after visiting international units carrying this type of work and adoption of their best practice code of conduct. Using this strategy has led to a lower harm rate than many of the published international literature. Animals are monitored carefully in the post-operative period and additional analgesics administered as required. Through adoption of humane methods of minimally invasive practice, we have been able to further refine this system and now practice even less traumatic surgery. Hence while the models described in the literature have utilised large abdominal incisions, we now principally practice a minimally invasive (keyhole) approach to the abdomen through a much smaller transverse incision, which also necessitates a smaller shaved area on animals, thereby further reducing trauma and any possible distress. This has developed through our experience in human surgery with an increasing trend towards minimally invasive practice where possible.

Project 30	**Engineered mice for gene function analysis**
Key Words	cancer, gene function, mouse, embryonic stem cells, embryonic development, developmental defect
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;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

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

The human genome contains 20,000 genes, but we know the role of less than half. Studies in humans have identified many genes that may be involved in disease, however in most cases we lack formal proof. Studies in a related mammal, the mouse can reveal both the normal function of these genes and their role in disease.

Although at first glance mice appear to be very different from humans, they have the same organ systems (brain, liver, lungs, heart, blood vessels, muscles etc). They have similar numbers of bones, though the size and shape are different and they show similarity in their reproductive cycles with females having a four day menstrual cycle. Mice also suffer from many diseases, just like humans, for instance they develop cancer and they can get heart disease on high fat diets.

These similarities validate the use of the mouse as a model for human disease. The parallels between the two species are also reflected in their genomes which are approximately the same size, contain the same number of genes and for most genes there is a one-to-one relationship between the human and mouse versions.

The mouse can be genetically manipulated, which offers the chance to model some of the genetic changes found in humans with severe diseases. We can also substitute mouse genes with the human version.

The overall goal of this project is to functionally analyse genes. In one aspect this involves destroying the function of specific genes and/or replace regions of the genome containing several genes with their human counterpart. We do this by making the genetic alteration in embryonic stem (ES) cells and we use these to make mice which transmit the modified gene(s) to their progeny which we inter-cross and examine. In some cases we replace the mouse version of a gene or several genes with their human counterparts, by doing this we can understand the importance of differences between the mouse and human versions.

Sequencing the genomes of human cancers has identified many possible cancer genes, but in many cases we are not sure if these genes cause cancer or were accidentally damaged in a cancer. To distinguish those genes which drive the cancer process from bystanders we can make mice which replicate the genetic alterations found in human cancers and study then to see if this causes cancer or not. In another aspect of our work we make large numbers of different genetic alterations in the cells of certain organs, for instance the intestine or liver using a mobile piece of DNA known as a transposon. We jump these bits of DNA around in the cells in the organs of a mouse and if they land in a gene which subsequently causes a cell to grow into a cancer, we can identify the damaged gene and prove that this causes cancer. With this knowledge we can design targeted therapies to kill the cells with this damage and thereby show how drugs can be targeted to control similar cancers in humans.

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)?

One objective of this project is to identify the function of genes. Because we can't experiment with humans in many cases we can't get mechanistic insight into a disease. This knowledge can have significant benefits; for instance it may directly identify and validate drug targets which can be used by pharmaceutical companies to develop interventions in the disease process. If drug development efforts is successful this will lead to improvements in human health. Another objective is the identification and validation of cancer genes. This is a starting point for elucidating the mechanisms by which they exert their effect. In some cases this suggests potential drugs which can be used to treat the cancer.

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

This project will use mice only, approximately 19,000 per year for five years. Most of these mice will be genetically modified and around 50% are used in straight forward natural breeding programmes to generate mice for future experimental use. Overall around 6,000 per year will be used for experimental studies.

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?

Most of the mice used in this project will be used for breeding and although they will carry one or more genetic mutations, they will not suffer any harm from these because they will also carry a normal copy of the gene. Therefore most of the mice will not suffer any adverse effect, other than that required to obtain a small sample

for genotyping. Between 5- 10% of the mice will have mild to moderate adverse effects from their genetic alteration and some mice may have developmental defects or they may develop cancer. In all cases the impact of the genetic lesion and the cancer will be strictly controlled to minimise the number of mice impacted, the duration and extent of the suffering. All mice will be humanely killed at the conclusion of study.

Application of the 3Rs

Replacement

Making and studying a mouse which lacks a gene is the accepted method to discover its function. If we wish to understand the role of a gene, a mutant has to be generated and examined in the context of the whole organism. It is not possible to recapitulate complex physiology or developmental processes in simple systems because the interactions of multiple different cell types and tissues can't be properly reconstructed. Therefore techniques such as cell culture and in vitro studies cannot be used.

The technology to identify cancer genes using human tumour samples has progressed markedly in the last five years. This has led to identification of many genes which are mutated in cancer. However, the numbers of mutant genes in most cancers is so large that it is very difficult to distinguish those which are causing the disease from those that are altered as a consequence. Some aspects of cancer can be modelled in a dish of cells, but to properly understand a cancer we need to study it when it's surrounded by normal cells in different sites in the body.

We always consider **replacement** and where there are alternatives we use them. For instance we conduct genetic screens in cultured cells to avoid animal usage, although invariably we need to validate findings in mice. Examples of this include screens for genes required for infection. In some cases these alternatives provide all the information we need, while for some experiments work in cell lines is a filter to select a smaller number of candidates for focused animal studies.

Reduction

The numbers of animals used for this project are based on 30 years of experience in generating mice with altered genes. We will continue to refine these methods, for instance by extensive pre-screening before starting any mouse work. We avoid large numbers of mice in breeding programmes by isolating and further manipulating ES cells, rather than breeding thousands of mice.

One of the most important aspects in assuring minimum numbers is careful planning of experiments to generate just the right amount of data and technical competence. This will insure that the goals are reached with the absolute minimum numbers of mice. We will strive to maintain highly skilled personnel on this project

Finally, data tracking and integrity are important aspects in reducing animal. The animal tracking data base at the animal facility greatly facilitates this effort. We publish our findings in open access scientific journals and share mouse lines and data with other researchers.

Refinement

The mouse was selected for this project because genetic technologies have been developed in the mouse over the last 35 years to an extraordinary degree of sophistication and efficiency. This is based on the Nobel Prize winning embryonic stem (ES) cell technology but is also supported by the sequence of the mouse genome. The refinement of the experimental design and techniques mean that many fewer animals will be needed compared to reaching the same goals in another species.

The other genetic changes contemplated in this project require that mice are unaffected by these additional genetic changes. Where possible we will search databases to ascertain the expectation for each gene that we manipulate before an experiment is conducted and if it is likely to be deleterious we will refine how it is manipulated to avoid welfare issues.

We maintain animal lines in a manner that does not produce mice with severe physical traits. We use the tissue obtained from ear-clipping individual animals for identification purposes for genotyping so that we avoid taking additional samples for this purpose when we can. We use technologies to limit any potential changes in genes to particular tissues which reduce the possibility of adverse effects.

Project 31	**Studying the origins of cancers from stem cells**	
Key Words (max. 5 words)	Paediatric, adult, epithelial, cancer, biology	
Expected duration of the project (yrs)	5 Years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our main objective is to understand the earliest events that drive normal stem cells to become malignant (cancer initiation). Better understanding of this process could lead to new approaches for early cancer detection and diagnosis, as well as novel therapies. Importantly, understanding how these are similar or differ among cancers may also provide fundamental insights into the origins and basis of different cancers.	
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 project holds great promise to make fundamental and much needed progress in advancing understanding of the origins, biology and treatment of adult and paediatric epithelial cancers. The benefits of this project are numerous and include, but are not restricted to: (i) advancing the knowledge of a variety of epithelial cancers (ii) provide insight into difference of neonatal and adult stem cells.	
What species and approximate numbers of animals do you expect to use over what period of time?	We will be working with mice and expect to use around 6300 mice over the licence period of 5 years.	

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?	Expected adverse effects are related to tumour growth in the animals and include loss of 15% body weight, and limited normal behaviour. Animals in distress will be humanly killer. All procedures are moderate in severity.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Because our approach requires the use of specific cancer-susceptible cell types at specific points in development, this is currently only possible by using live animals that fully recapitulate the complexities and cell populations present in development. Non-animal models cannot imitate the complex human or animal body. The advancement of knowledge and development of concepts to improve human and animal health and well-being requires the use of living animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use in vitro methods were possible to limit the number of animals required for the in vivo investigation stage.
3. 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.	Mice are the ideal species for our experiments: their lifespan (approx. 2 years) allows us to investigate tumour development over time and the scientific community has a range of techniques to manipulate the mouse genome allowing us to answer questions regarding tumourigenesis and tumour biology. We will minimise the animal suffering by monitoring the tumour growth and ensure it does not extend beyond the maximum permitted size/load. The surgeries will be performed under published best practise guidelines or where we have modified these to reduce suffering further.

Project 32	**Xenopus as a model for development and drug discovery**	
Key Words (max. 5 words)	Xenopus, developmental biology, Cancer, Stem cell biology	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	A human embryo starts off as a single cell after the egg is fertilised by the sperm. As this cell divides it develops into a multicellular organism containing lots of different types of cells, which give rise to the heart, eye, muscle, bone and other types of tissue. We are interested in how these different tissues arise from that single cell during development. Two of the major things cells in a developing organism need to do is to firstly, communicate to each other so as to tell each other what they should become i.e. a heart cell versus a liver cell and secondly, be able to move relative to each other to generate the specific shape of the tissue and animal they are going to become. When developmental processes such as these go wrong then congenital defects can arise examples of which include heart problems or spina bifida. Many of these developmental processes have also been shown to play a role in the adult and when there are problems can give rise to cancer and inflammatory disorders. It is difficult to study development in humans as the embryo develops inside the mother and gestation can take up to 9 months. Scientists therefore use animal model systems such as Xenopus (the African claw toed frog), which are easier to study to look at	

	developmental events. In addition in the drug discovery pipeline a new drug's potential toxicity is tested at the whole animal level in mice. This uses a lot of mice and is costly. We are developing protocols that would use frog embryos to test toxicity and so flag up harmful/toxic compounds at an earlier stage in the drug development process.
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)?	Knowledge gained from our research can be applied to human development especially as over the last 100 years it has been clearly shown that many developmental processes are conserved between humans and the rest of the animal kingdom. In addition our Toxicology research may lead in the long term to fewer experiments on higher vertebrates such as mice and rats as we will identify and flag potentially toxic drugs earlier in the drug development pipeline.
What species and approximate numbers of animals do you expect to use over what period of time?	We are using the tadpoles of Xeriopus laevis (the African clawed frog) and Xenopus tropicalis as a model organism. We use Xenopus because the females can be induced by a simple injection to lay eggs all year around when kept in an ideal environment and this provides us with a source of embryos (frog spawn). During the course of a year a colony of approximately 100-150 frogs will be induced to lay eggs 2-3 times, The better the quality of care of the frogs the better the quality of the eggs/embryos they produce. To therefore minimize having to repeat experiments it is essential to have healthy animals. The Xenopus embryos develop outside the mother and they are large which makes observation and manipulation relatively easy. Tadpoles have hearts, brains, eyes and kidneys just like humans. In fact a tadpole with a fully functioning heart, which can be observed beating using non-intrusive methods, develops from a fertilised egg within 4 days. Embryos produced will be used to study questions of cell communication and migration using a variety of methods. Therefore, what we learn in tadpoles about basic cellular and molecular developmental mechanisms will enhance our understanding of development in humans especially with respect to abnormal or disease situations.
In the context of what you propose to do to the animals, what are the expected adverse effects and the	The severity of the procedures on the female Xenopus and male Xenopus during induction of laying of eggs and fertilisation of the eggs is mild. When used for natural mating the females and males can be reused

likely/expected level of severity? What will happen to the animals at the end?	after a suitable recovery time (minimum 3 months) They can be used in this way multiple times until they reach an age when they no longer produce eggs of a suitable quality. At this time they are euthanised.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The purpose of the proposed work is to obtain fundamental knowledge on the development of a vertebrate organism (objectives 1-3). This requires working with the whole organism. With respect to our chemical genetic screens the use of the whole organisms helps with identifying compounds that produce a desired effect and at the same time screens out those molecules that have toxic or other effects. This is generally not the case with screens involving biochemical or cell based assays. Zebrafish are an equivalent model system used for chemical genetic screens. However the frog is over 90 million years closer to mammals on the evolutionary scale. Its organs are more similar and it has lungs and limbs, which fish do not. There are therefore potential advantages in carrying out screens on tadpoles compared to fish. We believe that screens with Xenopus will complement those that are also carried out in zebrafish. At the same time such high throughput screens that we carry out cannot be done on any higher vertebrates such as chick or mouse
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our Xenopus facility is designed to keep the frogs as healthy as possible. The healthier the frog the more likely she will produce good quality eggs. if we can be sure of obtaining good eggs wecan reduce the number of females we inject for each experiment. We are continually looking for ways to improve the care of the frogs in a cost effective manner. The Xenopus community in the UK and the rest of the world routinely discusses issues of care and welfare for the animals at meetings such as the annual British Xenopus group and once a year for the International Xenopus meeting or Xenopus P1 meeting in the USA. Web sites such as http://www.xlaevis.com/ also provide updated information.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	We mainly propose to use Xenopus laevis for our experiments as they are a well characterised and well used model organism for vertebrate development. They are relatively easy to keep and the adults are

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	subjected to only mild procedures. For some of our experiments Xenopus tropicalis will be used in the future. This is because Xenopus tropicalis is currently being increasingly used as a model genetic organism. As the embryos are half the size of Xenopus laevis embryos, which will be importantfor large scale high throughput chemical genetic screens. Both species have now had their genomes sequenced providing much valuable information.

<table>
<tr><td>Project 33</td><td>Hypoxia and Angiogenesis in Cancer Therapy</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Hypoxia, Angiogenesis, Cancer</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The purpose of this programme is to develop new treatments for human cancer, targeting angiogenesis (formation of new blood vessels) and changes that occur in cells in response to low oxygen (hypoxia), such as metabolism (how a cell or tumour makes its energy).

Angiogenesis is a key pathway for promoting tumour growth and its spread to other areas within the body (metastasis). Many current therapies target and block the function of one of the well-known proteins that drives angiogenesis and therefore metastasis. Unfortunately, these drugs can only improve the overall survival by a few months as many tumours become resistant to the drug treatment.</td></tr>
</table>

	Although blocking angiogenesis can kill tumour cells by reducing the delivery of nutrients and oxygen to the tumours – this in turn will increase hypoxia in tumours. Some tumour cells can adapt to this. Hypoxia can cause the tumour cells to be more aggressive (for example invoke a more metastatic phenotype) and they eventually become more resistant to current therapy by changing the metabolic and inflammatory tumour profiles. Many tumours contain areas of hypoxia even before blocking angiogenesis. Tumour hypoxia correlates with poor patient outcome to all types of treatment. We therefore want to discover key pathways responsible for angiogenesis and tumour cell survival/growth metastasis under hypoxic conditions and test their effects in combination with current therapy targeting angiogenesis in various animal models. Importantly we will see if these therapies can enhance the effectives of well established treatments such as radiation and chemotherapy.
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)?	Overall this licence will enable us to discover new pathways that are key to the progression of cancer as well as understand more about the mechanisms involved in cancer cells being able to adapt and survive various environmental stresses (e.g. hypoxia, acidosis, reduced nutrients). All together this knowledge will enable us to identify and design more relevant therapies that successfully target the pathways and overcome resistance mechanism(s) to current drugs used in the clinic. New findings will be published in academic journals and presented to other investigators at conferences, thereby aiding the research of other scientists. The results, particularly of synthetic lethality, in

	combination with drugs that are clinically available, will be translated into clinical studies in an appropriate setting. For example, drugs that are only in phase II and not licensed, cannot be used in early disease, but could be used in metastatic disease in patients. We have great experience in this area and have completed over 16 trials now whereby biopsies need to be taken before and after treatment. This is funded in the majority by CRUK and the University, with a large number of investigation-led studies assessing new treatments. We have collaborations with many pharmaceutical companies, through Cancer Research UK, which allows combinations of two drugs from different companies to be utilised. So, there are now several routes forward to take in the clinic, combinations of drugs from different companies, and also with radiotherapy, as we have state-of-the-art radiotherapy equipment here now to be able to give highly focused fields and treat small volumes of disease in vital organs. This is one of the reasons we have particularly wanted to bring radiotherapy into our protocols because of the recognition of a new management approach to oligometastatic disease where patients have a metastasis in brain, liver, lung, pancreas, bone, but a low volume and they can be potentially cured, even though they have metastasis, by giving radiotherapy to the sites. We are a National leading centre in this area of research. So it is particularly important to try and synergise with radiotherapy because we can give high doses in small areas and the vessels seem to be one of the major ways of dealing with this as does changes in metabolism. So our PPL is strongly linked to the research capabilities and interests of our Department

	and the CRUK
What species and approximate numbers of animals do you expect to use over what period of time?	We plan to use the following animals over 5 years Mice = 3400 (including 800 for breeding purposes) Rats = 350 Zebrafish = 20,000
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?	Most of the mice and rats will be implanted with tumours that grow under the skin up to a set limit, which will not impede their movement. The animals will then be treated with experimental drugs, radiation, chemotherapy or both. Animals may be injected with tumour cells plus stromal cells and the effect on the spread of cancer cells within the animals will be assessed. In some experiments we will excise the primary tumour and follow up the animal to see if we can block the growth of spontaneous metastases. Additionally, we will use transgenic mice to express proteins (or switch them off) that then lead to the tissue-specific induction of tumours. The animals will then be treated with experimental drugs, radiation, chemotherapy or both. We expect in some cases that animals will lose weight, can lose appetite, can experience mild diarrhoea, can get fluid in their guts and the surface of skin above growing tumours can become mildly ulcerated. Using imaging techniques to monitor changes within the tumour will provide vital data on the effect on the growth and progression of the tumours. We can also assess how well the drug affects its target (drug efficacy) in these assays. At the end of the experiments the animals will be killed and the tumours will be examined to confirm changes in the blood vessels within the tumours, tumour proliferation, cell death

	and metabolic changes. Blood samples will be taken to so that we can assess changes in proteins levels and how these changes correlated to the effect on the tumour growth.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have developed assays to culture cancer cells in the lab under tumour-like environmental conditions, and also various angiogenic assays where we grow endothelial cells We are still limited in the ability to model the complex dynamics between stroma, tumour and blood flow that occur within humans, and evaluating the efficacy and metabolism of drugs is only possible within an animal setting.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In order to minimise mouse use and experimental severity, in vitro experiments that model aspects of angiogenesis will be performed in the first instance. Experiments in mice and rats will only commence when strong in vitro data is present and experimental design ensure that the maximum amount of data can be obtained with the minimum animal numbers, with minimal invasiveness. Imaging techniques to enable us to monitor effect on tumour growth and vasculature over time in the same animal will allow us to achieve this, and significantly reduce animal numbers. Using fluorescently tagged proteins in transgenic models, e.g. ATG8 to monitor autophagy, will allow much more accurate assessment of tissue specific responses and their quantitation. This is compared to the variability of using antibodies. Although it would not allow non-invasive imaging, it will reduce the number of animals needed because of its greater reliability.

3. 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 fish will help us to evaluate the effects of modifying targets on angiogenesis and tumour progression and thereby act as a screen prior to work with mice/rats. Immuno-deficient mice are preferred strains as they are capable of sustaining human tumour growth and tolerating therapy over longer periods of time. The use of transgenic mice will allow us to assess changes in tumours grown under the skin, or at same sites where tumours develop in humans (e.g. breast tissue) and also metastatic models to assess spread of tumours – all of these are generally considered to be a better model of human disease. Non-invasive live imaging of both tumour growth and transgene expression will provide early endpoints to determine the success of modification or activity of agent. Pilot studies will be carried out using small numbers of animals to assess toxicity and efficacy of drugs before commencing larger studies. We have strict welfare boundaries in place to ensure the wellbeing of the animals is always promoted and only those that are experienced in the techniques required will handle animals. Maintaining good records of welfare changes, in line with outcome of experiment, will ensure future experiments are designed appropriate to minimise welfare impact and also provide maximum amount of scientific data.

Project 34	**Mechanisms of Metastasis**	
Key Words (max. 5 words)	Metastasis, myeloid cells, coagulation, extracellular matrix	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objectives of this project are to gain basic scientific understanding of the mechanisms underlying the spread of cancer, especially to the lung and liver, the two most common sites for human cancer metastasis. Currently metastatic lesions in patients with cancer often fail to be successfully treated resulting in outcome failure. It has become recognized recently that the interactions of the metastatic cancer cells with the normal cells and tissues at the metastatic site often drive the development, persistence and the resistance to therapy of metastasis. These experiments are designed to elucidate these interactions and the mechanisms by which they support metastasis.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	As indicated above, much about the growth and persistence of metastatic cancer lesions is not understood. Here we expect to add to the scientific understanding of metastasis and hope to suggest therapeutic strategies. Identification of the host cell	

project)?	types and proteins that the normal cells make in response to the metastatic cancer will reveal some of the mechanisms through which the cancer cells subvert the normal host processes to facilitate metastasis. Additional understanding of the metastatic process, especially as involving the lung and liver will emerge from these studies. Further therapeutic strategies and diagnostic markers for metastasis may also be a result of these studies. Given the current intractability of metastasis to therapy, inroads into therapeutic targets from this type of research could greatly alter and improve approaches to cancer therapy.
What species and approximate numbers of animals do you expect to use over what period of time?	All of our work will be performed in mice. We expect to use up to 22,000 mice over 5 years, 16,000 of these for breeding.
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 bulk of the experimentation will involve induction of metastatic cancer by injecting cancer cells into the blood stream or allowing the cells to spread naturally from a primary tumour which may be a implanted under the skin or into the breast tissue, the liver or the colon. Inhibitory drugs may be injected or administered in the drinking water. We expect to perform the majority of the experimentation while the tumours are small so that systemic effects of tumour burden will be minimal and the mice will suffer minimal discomfort. To examine the response to treatment, metastatic growth may be followed by repeated imaging under anaesthesia. In some cases the mice will be irradiated and reconstituted with bone marrow. Whilst these transplants take hold the mouse will be deficient in various blood elements. They may experience weight loss or diarrhoea. They will be closely monitored and either treated with antibiotics or humanely killed if they become unwell. All mice will be humanely killed at the end of the study.
Application of the 3Rs	
1. Replacement	Currently it is not possible to simulate cancer cells in the environment of the lung or the liver in non-animal

State why you need to use animals and why you cannot use non-animal alternatives	settings. While several components can be mixed in tissue culture, this type of experiment omits many of the key constituents that include many different types of immune cells, blood vessels, fibroblasts and extracellular molecules including proteins that aggregate to form the extracellular matrix. Once specific interactions are identified, some of their characteristics can be simulated in tissue culture as well as preliminary studies demonstrating inhibition, and these experiments help inform our work. But in the end, only the in vivo experiments have the capacity to put the multiple features into play and the capacity for testing clinical strategies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use statistical calculations based on prior experience to minimize numbers that would be expected to give rise to an experiment that has statistical power. We also attempt to obtain the maximal amount of information from every experiment to reduce numbers. Finally by using imaging, we can follow the same animal with time enhancing statistical power and reducing the need for additional animals at each time point. Pilot experiments also inform as to the conditions and timing that should lead to the best results from the fewest animals.
3. 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.	Currently murine models for metastasis are the gold standard for the field. Hence these are the most refined as they are essentially the only models agreed in the field. In human cancer, a primary tumour forms followed by spread to distant sites. In the mouse, this pattern only works for lung metastasis. Metastasis to other sites from this type of model is very rare, too rare for experimentation. Further the metastases tend to be sporadic. Thus this model can be used in some settings but when more detailed timing is needed a variation is to inject cancer cells into the blood stream so that they lodge in the organ of interest the lung, followed by growth. This model omits the step of blood borne dissemination but provides more precise timing and numbers. Hence neither model is more refined, but the choice depends upon the question asked. With

	liver metastasis, there are no models of primary tumours that reliably work under the experimental conditions we wish to investigate so that injection into the blood stream that drains into the liver is the only available model. To minimize welfare costs to the animals, we monitor them closely. We seek to perform experiments with the lowest feasible tumour burden, i.e. to keep the tumours and metastases as small as possible. We also seek to use imaging to follow tumour burden in many cases when it cannot be directly observed. We will use anaesthesia where possible, to reduce temporary discomfort or stress during a procedure and pain relief will be supplied as necessary when animals receive surgery, which will be performed under aseptic conditions. Pilot experiments will be performed on small numbers of mice to study the growth and behaviour of new tumour systems so that action points, monitoring schemes and humane end points can be defined before larger experiments are commenced.

Project 35	**Induction of Anti-tumour Immunity**	
Key Words (max. 5 words)	Cancer, white cells, immunotherapy	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall aim of this project is to improve our understanding of the relationship between the immune system and cancer and to use this information to design new immunotherapies.	
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)?	The design of new immunotherapeutic approaches to be tested in patients with cancer.	
What species and approximate numbers of animals do you expect to use over what period of time?	A maximum of 5,400 mice will be used over 5 years.	
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	Animals used in experimental procedures will be handled frequently (approximately three times per week). During these times animals will be injected and / or monitored for tumour burden. Through good handling techniques, distress caused to the animal	

happen to the animals at the end?	from being restrained will be minimised in terms of time and discomfort (a single animal will typically be restrained for less than 30 seconds). Mice will be monitored for tumour burden frequently and tumours will be scored for size, position and ulceration. Should tumours limit mobility, appear ulcerated or reach a maximum permissible size, animals will be killed by a schedule 1 method. Based on working with mouse models of tumour growth since 1999, it is expected that for the majority of animals used in this project, the severity level will not exceed “Mild”.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Interactions between the immune system and developing tumours depend on the integration of signals from a number of immune cell types and immune mediators. With this in mind, an animal model is necessary as there is currently no way to recreate these *in vivo* conditions in an *in vitro* setting.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments will be designed based on previous findings which will inform the minimum numbers required to achieve statistically significant findings. This project will make use of a number of different imaging modalities for measuring tumours which are not visible to the naked eye. This will enable us to carry out longitudinal analyses of tumour growth in individual mice thereby significantly reducing the numbers needed to assess the impact of a given immunotherapy on tumour growth over time.
3. 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 provides an excellent model in which to study the immune system. Mice are well characterised immunologically, and their immune systems closely resemble those of humans. Tumours will be induced either through injection of carcinogens or with tumour cell lines. Both procedures involve minimal restraint and little discomfort to the animal. Tumours are monitored frequently either by imaging or palpation allowing us to kill animals by Schedule 1 methods before tumours

	cause pain / discomfort.

Project 36	**Modelling cancer biology and therapy in mouse**	
Key Words (max. 5 words)	Breast mammary cancer metastasis xenograft	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	1. To identify genes responsible for the progression of breast and other cancers. 2. To demonstrate whether alteration of these genes (or gene products) has a therapeutic effect which could, in the future, be utilised in the clinical setting for humans. 3. To generate data to support the clinical translation of two putative anti-cancer agents previously identified by the host laboratory. 4. to test whether any of these genes have the potential for early detection of metastatic tumour cells by non-invasive imaging modalities, including MRI and PET.	
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)?	Two new anti-cancer agents are currently in the process pre-clinical development with the view of performing first-in-man clinical trials of these within the timeframe of this project.	
What species and approximate numbers of animals do you expect to use	Approx 2500 genetically altered mice.	

over what period of time?	
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?	Mice will either be genetically predisposed to developing cancer or will be transplanted with cancer cells and then the effects of anti-cancer agents or other types of intervention will be assessed. This will result in a gradual increase in tumour burden in one or more sites within the body which may lead to visible signs of morbidity. These procedures are considered moderate as the animals will be carefully monitored to prevent onset of severe adverse effects. The majority of mice will be killed by schedule 1 method, less than 1% will be exsanguinated under terminal anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are looking to prevent the spread of cancer around the body. This is a complex process that requires a living (mammalian) body with all its constituent organs to study this process and to determine if certain interventions are able to suppress this.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Mouse numbers are minimised by acquiring experimental animals from commercial sources rather than breeding them in-house. Mice will be imaged (scanned) under general anaesthesia repeatedly over several weeks rather than killing mice at timepoints to investigate tumour burden.
3. 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.	Mice are chosen as they are the most genetically tractable animal species, they possess mammary glands (we study breast cancer predominantly), there are a variety of immune compromised strains for transplant experiments there is a large body of knowledge on the physiology, histology and molecular biology of the mouse, they have a relatively short life-cycle and high fecundity and mice are generally regarded as being of lower sentience compared to other mammals such as the primates. Measures to minimise welfare costs to animals include: restricting genetic alterations or site of tumour incidence to specific tissues through

	conditional transgenics; the early detection of tumour burden through the use of scanning techniques to detect internal tumours; and a cumulative morbidity scoring system which assesses multiple parameters of tumour-related morbidity

Project 37	**Establishment of Patient Derived Xenografts from Biopsy Samples**	
Key Words (max. 5 words)	cancer, tumour, biopsy, tissue generation	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The main objective of this project is to determine how we can use patient-derived tumour tissue with other human cells and/or molecules that influence the behaviour of cancer cells (including their growth rates and ability to spread to distant sites) to ensure that tumours generated remain as close to the patient tissue as possible. Having such models available is important in order that such tissue can be used in our experimental programmes aimed at developing better methods for detection and treatment of cancer. Our aims are to: OBJECTIVES 1) determine the cells and substances which need to be added to patient tissue to generate clinically-relevant tumours in animals, maintaining the behaviour and molecular characteristics of the original patient tissue and ensuring the best chance of tumour growth.	

	2) use the tissues expanded in the animals to establish *in vitro* (laboratory-based) cancer models using tissue from objective 1 which, again, include important supporting cells and molecules in which cells are not grown on plastic dishes, but within a more tumour-like 3-dimensional scaffold. 3) use these non-animal models to screen new agents/drugs for detecting and treating cancer in a more clinically-relevant setting prior to screening in animals 4) directly transplant tissue into experimental animals, under separate licence authority held at this establishment as part of our experimental programme, to screen new agents/drugs for detecting and treating cancer in animal models 5) secure the models for future use, to minimise the number of mice required to maintain the line 6) identify optimal method of cryopreservation for effective storage of tumour tissue.
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)?	The primary benefit would come from the supply of tissue to further our work in the development of superior cancer models, more representative of the patient's tumour, incorporating mixed human cell types derived from patient tissue and established in cell culture systems which are more representative of the patient's tumour in terms of biology and response to treatment. These models can then be used in our drug development programme which could lead to new treatments being made available in the clinic, and will also mean we will use fewer animals in the future as the models can be used instead. We will also be able to bank down this valuable tissue as a resource for future use by us and by our collaborators.
What species and approximate numbers of animals do you expect to use over what period of time?	Immunodeficient mice, maximum of 500 over 5 years

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?	Animals are being implanted with small fragments of human tumour tissue (removed by surgery from patients during the normal course of their treatment). The implantation procedure is a non-surgical process, whereby the tumour fragment is placed in a small pocket made under the skin using a large needle under local anaesthesia and, therefore, no adverse effects are expected. Tumours could ulcerate before reaching maximum permitted size (1.2cm mean diameter), or may hinder movement depending on position. Both circumstances will mean that the animal would be humanely killed. Hormone supplementation, if delivered by slow release pellets (implanted in the same way as the tumour fragments), or injection of cells into the tumour may cause broken skin, so if any skin reddening or breakdown occurs, the animals will be humanely killed and, in the case of the hormone delivery, giving it in food will be the route of choice wherever possible. All animals will be humanely killed to harvest tissue at recognised end points. The level of severity is mild.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	While our experimental programme is focused strongly on in vitro modelling, it is currently not possible to generate a sufficient amount of cells required to develop these models directly in vitro from patient biopsy samples. Therefore, mice are still required as a vector for this initial tissue establishment and maintenance, until sufficient stock tissue has been generated for in vitro experimentation and investigation, and for banking down for future use.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals used per sample is dependent on the amount and quality of tissue delivered from the operating theatre. Typically, after preparation, the tissue will be implanted into 1 or 2 mice, but never more than 3. Once the initial samples have grown they will be transplanted on into the minimum number required for tissue generation and examination by e.g. histology, immunohistochemistry.

	Typically, this will be a further 2 animals but exceptionally, may be up to 4 depending on the amount of tissue required. Therefore, group size is sample size driven rather than to provide statistical significance, so we will always use the minimum number of animals needed for the amount of tissue required.
3. 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.	Mice are the lowest species in which a genetic mutation has produced an immunocompromised (reduced immune system) status which allows growth of human tumours in a clinically relevant setting. Also, the application of our experimental technologies requires a species with a similar body system to humans, and it must also be able to generate a sufficient amount of tissue. Experience has shown us which immunodeficient mouse strain is most appropriate for particular tumour types but we will always try to improve take rates by trying new strains which may become available. Our procedures have been refined to minimise suffering by using local anaesthesia and a trochar (large bore needle) implant method, rather than surgery and, in the case of hormone dependant tumours e.g. breast and prostate, we deliver the necessary hormones by incorporating them into the diet where possible.

Project 38	**Pancreatic Cancer: Biology and Therapy**	
Key Words (max. 5 words)	Pancreatic cancer, Pancreatitis, Therapeutic targets	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall goal of this project is to identify and characterise the genetic events and molecular pathways involved in the initiation and progression of pancreatic cancer, and to use this information to discover effective treatments and improved diagnostic tools. Our plan is to improve current and develop new mouse models that can be used to study the molecular mechanisms required for pancreas cancer initiation, progression and metastasis. We will also use mouse models that faithfully recapitulate the clinical, pathological and pharmacokinetic features of the human disease to identify therapies and diagnostics. We will employ in vitro approaches whenever possible to characterise molecular pathways involved in the development of pancreatic cancer. However, tumour biology is profoundly influenced by its environment within the body and tissue culture systems are unable to mimic this complex interplay. Moreover, these animal models will allow us to investigate critical	

	questions about this disease and the effectiveness of novel therapies which are not possible to address in patients. Planned procedures involve the use of mice designed to develop tumours, typically pancreatic tumours, but some experiments will use mice with external subcutaneous tumours or mice that have pancreatitis. Procedures we intend to use include the administration of potential therapeutic agents and imaging. As in human patients with pancreatic cancer, we expect that there will be an impact on the overall health of some the study mice. However, the identification of effective therapies and diagnostics requires accurate translational models that mirror the clinical features of the human disease. Every animal will be monitored for signs of distress and pain and will be given analgesia or killed as appropriate. We will consult with experts, for example in small animal imaging, to ensure that the experiments we carry out are optimised to collect the maximum amount of information with the least suffering to the animal.
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)?	Pancreatic cancer is an almost uniformly lethal disease with a five year survival rate of less than 5%, making it the fourth most common cause of cancer deaths. The majority of patients are diagnosed with inoperable disease for which there is no currently available effective therapy. These grim statistics reflect our inability to detect early stage disease and its highly metastatic propensity and innate resistance to current chemotherapies. We expect that this project will provide novel insights into genetic and cellular events involved in the initiation and progression of this disease from a preinvasive and potentially curable phase, until an invasive and lethal stage. This knowledge will not only be of interest to scientists studying tumour bio1ogy, but could also lead to the discovery of new strategies for treating pancreatic ductal adenocarcinoma, which is among the most lethal human cancers.

What species and approximate numbers of animals do you expect to use over what period of time?	The mouse is the experimental animal of choice for this project. We plan to use around 1,750 mice over 5 years.
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?	Around 25% of the mice produced in this project are expected to develop pancreatitis and/or internal tumours. These mice are expected to experience a moderate severity level. However, it is not possible to fully predict the nature or severity of any potential defect and all types of mice will be closely monitored for possible side effects. Animals exhibiting any unexpected phenotype likely to exceed a moderate severity will be killed. For breeding, we will aim to utilise animals that exhibit no or only mild phenotypes. However, should the development of more harmful phenotypes be necessary to allow maintenance of certain lines of scientific interest, animals will not be allowed to exceed the limits set in the protocol. Additionally, some procedures will be used in this project will involve surgery and/or the administration of substances, agents, cells and tissue fragments. Mice will be monitored for signs of distress or pain, and will be given analgesia or killed as appropriate. A small group of mice under the protocols 5 (10) and 6 (40) may reach a severe level of severity. Mice from these cohorts will be closely monitored and, according with the NVS, they will be killed whether they undergo excessive discomfort. The rest of the mice on the project are not expected to experience pain, discomfort or distress considered to be of more than moderate severity.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The objective of this project is to model human pancreas cancer in order to understand the mechanisms underlying this disease and to use this knowledge to discover effective treatments and diagnostics. Tumour biology is profoundly influenced

	by its environment within the body. In vitro systems such as cell culture cannot mimic the interactions of tumour cells with and the influence of other cell types, such as connective tissue, immune cells and the vasculature. The effectiveness of potential pancreas cancer therapies can only be assessed in vivo. Moreover, animal models which reflect the pathological features of human pancreatic cancer are likely to predict clinical responsiveness in humans more accurately.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Many of the mouse models we plan to use harbour more than one mutation. To reduce animal numbers, the breeding strategies are designed to produce mice with the experimental genotype as efficiently as possible and, in most cases, appropriate control animals will also be generated from the same crosses. Additionally, we will keep stocks of frozen sperm and embryos so that if a mouse line is not continuously required we can avoid unnecessary breeding. We plan to collect tissue samples from experimental animals to generate a tissue repository that will be available for future in vitro experiments. We will plan experiments so that the minimum number of animals will be used without compromising the scientific aims of the study. Only if the therapy impacts some parameter of tumour progression, will expanded studies be designed to allow a statistical evaluation of efficacy. The number of mice required for an expanded study will depend on the impact of the treatment on tumour progression, but is usually in the order of tens of mice. We will obtain statistical advice in order to improve the design of therapeutics trials.
3. 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	Mice are a well-studied experimental species. They breed easily, there are many strains available, and the technology to genetically manipulate mice is well known. For these reasons, the mouse is the experimental animal of choice for this project. The mouse models we use to address each specific question are chosen to achieve the scientific aims with the minimum of animal suffering.

costs (harms) to the animals.	For therapeutic studies, we will perform pilot experiments with small numbers of mice to ensure that there are no unexpected adverse effects from the treatment and to ensure that there is sufficient evidence to warrant larger scale studies.

Project 39	**Colorectal cancer initiation and progression**	
Key Words (max. 5 words)	Colorectal, cancer, RNA, genes	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Colorectal cancer is the second commonest cause of cancer related mortality and there is a pressing unmet need for the delivery of new therapies for its treatment. Determining how tumours initiate and progress is critical for understanding tumourigenesis and guiding therapeutic development. The aim of this research programme is to identify potential therapeutic targets by determining the mechanisms important for tumour initiation and progression. Our preliminary data suggest a role for a process termed alternative RNA splicing during tumour initiation. Changes in RNA splicing are a poorly studied tumour mechanism despite them being ubiquitous in cancer. Additionally, large scale sequencing of tumours has identified 100s of genetic mutations in colorectal tumours. Again, the function of the vast majority of these mutations is completely uncharacterised. During this research programme we will use appropriate, well- defined mouse models to address 2	

	aims: 1) The role of alternative RNA splicing in cancer 2) The identification of novel cancer driver genes
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)?	Expected benefits for objective 1. My research has the potential to define a novel cancer mechanism. This will increase our understanding of how tumours form and spread and open new therapeutically exploitable opportunities. Expected Benefits for objective 2. The identification of novel mutations that drive cancer will provide great benefit to the understanding how tumours form and spread. It has the potential to identify mechanisms important for tumourigenesis. This in turn may lead to the development of novel therapeutic strategies for targeting colorectal cancer.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse 15000 5 years
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 primary adverse effects on the mice in this research programme are the development of intestinal/colonic tumours. When intestinal tumour burden is high in mice, mice become anaemic (as shown through paling of the feet) and begin to lose weight. In around 10-20% of mice rectal bleeding is expected. This occurs due to the presence of small colonic/rectal tumours and is well tolerated by the animals (they show no other obvious signs of distress). Close monitoring will occur after the observance of anaemia and/or bleeding and mice will be humanely culled when they show signs of moderate discomfort. This is described as three of the following symptoms - weight loss over 20%, subdued behaviour, stop interacting with their cage mates, anaemia, sustained rectal bleeding with visible blood on fur and sustained diarrhoea for over 72h. Other mice will be culled at pre-defined time points according to the experimental

	protocol at which time tissues will be taken for analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	As colorectal tumours are very complex and have a variety of different components e.g. tumour cells, immune cells, supporting cells (stroma) and blood supply, it is not possible to mimic these processes outside of the body. Experiments in animals better recapitulate these effects on how tumours grow and as such it is important to study it in this context. The involvement of the tumour support system and the immune system on cancer cell development and response to therapy is well documented and highlights the need to use mouse models for these studies. We, and others, have developed a number of advanced tumour growth techniques that do not require animals. These will be used in parallel to these studies thus reducing the number of animals used by focusing their use on addressing the key questions throughout the proposal. However, for the reasons outlined above, these models fall short of replacing the need to carry out these experiments in animal models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have carried out mathematical analyses to ensure we use the correct number of animals in our experiments. We seek to always use numbers of animals that are likely to give a meaningful result based on our extensive past experience and the use of these calculations. We ensure that any tissues generated from previous experiments are archived and stored appropriately therefore ensuring that repetition of experiments is not necessary.
3. 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.	We will use genetically engineered mouse models throughout this research programme. These models have been chosen because the mutations that they carry are those associated with the human disease allowing us to recapitulate the same genetic mutations observed in human cancer. No other models are currently available to perform this analysis. Where possible we use advanced ‘targeting’ systems to specifically target the tissue of interest thereby reducing off- target adverse effects in the animal. The

	group will ensure that all animals receive the highest standard of care, and appropriate social, environmental and behavioural enrichment will be provided. Close monitoring of tumour development will ensure animal suffering is kept to a minimum. We have vast experience of these cancer models and the clinical signs that develop; this allows us to kill animals exhibiting mild-moderate clinical signs at an earlier stage in the process. For all our studies we will refer to the Guidelines for the Welfare and use of animals in Cancer Research (Workman et al, 2010) and ensure best working practice.

Project 40	Model Systems to Improve Cancer Immunotherapy
Key Words	Cancer, Immunotherapy, Mouse
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;

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

This project focuses upon the development of immune-based therapies to treat cancer. Exciting clinical results of immune-modulating or -exploiting agents has demonstrated clinical benefit and durable remissions in certain tumour indications. These immune-based therapies include antibodies targeting immune system control mechanisms and adoptive T cell transfer where naturally occurring or artificially engineered tumour-specific T cells are infused into the patient where they can mediate a direct anti-tumour response.

Whilst highly impressive, it is clear that understandings of the mechanisms of anti-tumour activity are limited. The current approaches fail to deliver significant therapeutic response across all tumour indications. This licence seeks to develop the understanding of the immune cell system approach to tumour treatment further. The project aims to improve the therapy whilst examining the underlying immunological and biological factors that hinder the broader successful application of cancer immunotherapy.

The underlying purposes of this project are:

1. To optimise and develop cancer immunotherapy in order to provide the highest level of anti-tumour effect with minimal toxicity.

2. To understand the factors that blunt cancer immunotherapy and to develop solutions that enhances the effect of these approaches.

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)?

The key benefit of this project will be the pre-clinical development of cancer immunotherapies with the intention of delivering protocols for the early phase testing of these approaches in patients with advanced cancer. This builds upon our track record of delivering engineered and natural T cell therapies in trials. The work will also advance our understanding of which mechanisms the tumour employs to blunt specific immunotherapies. We can then develop modified or combine strategies that can deliver a broader anti-tumour effect, essential to the future development of the approach overall.

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

We expect to use up to 7,750 mice over five years.

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?

Adult mice are prepared (“conditioning”) to receive implanted / injected tumours by either the exposure to controlled levels of UV exposure or through administration of specific chemical agents, or a combination of the two, to deplete specific populations of circulating white blood cells prior to receiving tumour transplantation. Mice will then be selected for tumour implantation based upon the success of the conditioning. Implantation of tumour cells will be either by injection or implantation under the skin or injection into specific organs to mimic particular cancers. Either mouse or human stem cells may also be injected to provide a source of the white blood cells. Tumour growth will be measured by either the use of callipers or by non-invasive imaging under recovery anaesthesia. Pilot work using small numbers of mice will be undertaken in order to design a study in the optimal way and using the minimum number of mice. Therapeutic and experimental therapeutic interventions either given alone or in various combinations will be administered to the mice, either by mouth or injection, and their impact upon tumour growth and/or growth free time will be monitored. All mice will be very closely monitored throughout their treatment with clear cut-off points for culling the mice based upon humane endpoints. Due to the nature of the models the majority of the mice will experience the Moderate severity band. The project will aim to achieve the clinical endpoint with the lowest possible impact on the health of the mice. All mice will be killed humanely, at the end of the experiment. Potential side effects include, loss of bodyweight, body condition and hunched appearance. Reduced food and water intake and potential ulceration of tumours are also possible. All mice will be closely monitored and where there is persistence of adverse effects animals will be culled humanely, mainly by Schedule 1 method. On very rare occasion there may be a spontaneous death.

Application of the 3Rs

Replacement

At present, it is not possible to model the complex interactions that occur between the immune system and healthy and diseased tissues. There are no test-tube or computer models that can detail the complex phases of immune system induction nor able to model the array of cell-cell and cell-extracellular matrix that occur. However, currently available test-tube systems will be used to investigate the linear interaction of immune cell – target tumour cell prior to use in mice.

Reduction

The key effort on reduction focuses upon the use of pilot studies to inform the likely group sizes required to achieve a statistically relevant effect. We will also use in vivo monitoring to provide as close as possible to real-time data and thereby reduces the number of animals required per group to follow internal tumour responses.

Experiments will be designed to include as many test groups as possible thereby reducing the numbers of control groups that would be required over a series of smaller experiments. Statistical analysis between groups (such as analysis of variance) will be used to compare the results in the experiments (e.g. engraftment of immune cells, tumour growth over time). The statistical power of this work will be determined through pilot studies.

Where repeat experiments are considered essential, these will be performed using minimal numbers of animals and will be incorporated within a single experiment wherever possible to reduce the number of control animals being tested at any one point. Advice will be sought from on-site statisticians to help predict optimal group sizes and treatment regimens.

Refinement

The adult mouse is the primary species proposed for this work based upon:

- The mouse immune system has been intensively investigated and is the most advanced in terms of understanding.
- The necessary reagents are available and usually well tested which is not the case for most other species.
- Previous studies have used the mouse thereby ensuring a continuity of work.
- The mouse represents the lowest level of animal that accurately reflects the human system for immunotherapeutic studies.

To avoid opportunistic infections in immuno-compromised mice undergoing treatment that impairs immune function, animals will be housed in ventilated containers.

Orthotopic tumours will be monitored on a regular, sequential basis by in vivo imaging techniques.

Cancer immunotherapy seeks to drive tumour specific immune responses but given the similarity between tumour and healthy tissue, on-target effects frequently occur. Consequently, effective anti-tumour therapy may be associated with transient adverse effects such as the immune cells attacking the normal as well as the cancer cells. For the agents planned to be used within this project, no such adverse effects have been observed when given to mice. However, careful monitoring of mice by experienced staff members will be performed regularly to ensure that prolonged suffering is not experienced.

Wherever possible, analgesia will be provided to control adverse symptoms in mice.

Project 41	**Implantable Microsystems for Cancer Therapy**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our objective is to develop implantable biosensors that can be used to monitor a patient's cancer in real time and therefore it will be possible to adjust treatments accordingly. The ultimate aim is to improve cancer treatment efficacy and thus long term survival. We propose to do this through studying disease in animals with similar cancer biology, anatomy and physiology to humans – namely sheep.	
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)?	• We will identify the most appropriate and safest biosensor material for use in biosensors. (which could be used in animals or man) • The work will produce a validated sensor that could ultimately be translated into human medicine or veterinary medicine.	
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice initially to establish the biosafety of the materials to be implanted (6 month project using around 150 mice). Sheep will be then used to establish the efficacy of the biosensor in real time and under cancer treatment conditions (around 10-20	

	sheep over a 6 month period.
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 level of severity is mild to moderate and all animals will be euthanased at the end of the project. The mice will be subjected to tumour growth to a size where we can implant a small “blank” sensor. The size and shape of these make them similar to a needle injection. We anticipate therefore that the risk of adverse events is low and the level of severity is mild to moderate. The sheep will have naturally occurring lung cancer, which is due to a virus infection and occurs on british farms. The sheep in this study will have tumours but at a size which is insufficient to cause any breathing problems or other adverse effects. The sheep will have very small sensors implanted in a lung tumour and will then be exposed to radiation that mimics radiation treatment of cancer. Anaesthesia, surgery and irradiation involve potential risks of ill health for the animals. . However, we minimise these risks by ensuring that very skilled people are using methods with the lowest possible impact on the animals and any animal that is suffering will be treated or euthanased. Carefully chosen doses of radiation will be used which will also minimise this potential. We would consider the expected level of severity to be moderate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	• We have used in vitro methodology to take this research as far as is feasible. It would be impossible to answer all of these research questions in tissue culture or computer simulations. This is because we need to establish the safety and functionality of a sensor in 3 dimensions (in association with an intact tumour microenvironment) and in the context of a living animal. It will be possible to use tissue culture to identify some aspects of how cells respond to the materials that make up the sensor.
2. Reduction Explain how you will assure the use of minimum numbers	• Through using appropriate experimental design and statistical methodology to ensure we use the minimum number of animals required to answer the scientific questions. • We will utilize non-invasive ultrasound

of animals	identification of animals for this study so we are not subjecting inappropriate animals to procedures. • Animals will act as their own controls and we will also be following changes in the same animal over time without the need to use additional animals.
3. 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.	• We intend to use a mouse model in the first instance to establish the effect of implanting a "blank" sensor. This is to ensure the sensor components are themselves not toxic. This requires the use of a tumour in a living animal to offer the most appropriate system for predicting any potential effects in man. The mouse model we are using is predictable and reproducible and is therefore the most refined for this component of the study. Tumours will not be allowed to grow beyond 12mm diameter and no ulceration will occur. This model allows us to be able to answer the basic safety questions within this species without the need to go to a large animal model. • We have to go beyond this model to robustly evaluate efficacy and applicability in cancer, especially using a sensor of a size that would be inappropriate in a mouse model. For this reason, we believe that the sheep model offers an appropriate model system. Cancer in sheep offers an alternative to mouse models of cancer and as they share important characteristics with the corresponding human disease. • The delivery of the sensors is being refined in ex-vivo models (non-living tissues) prior to moving to live animals. • In terms of refinement, where possible we will perform procedures under one single anaesthetic and complete recovery will be ensured before any further procedures are carried out • We will use analgesia in any procedures likely to cause any discomfort.

Project 42	**Study and manipulation of immune-regulatory receptors to improve cancer therapeutics**	
Key Words (max. 5 words)	Cancer, Immunology, Immunotherapy, Checkpoint	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall goal of this project is to study and interfere with the interplay between the immune system, tumours and their particular microenvironment using mouse models of cancer that better mirror human malignancies. During tumour growth, the immune system makes several attempts to fight the developing cancer but fails due to the ability of tumour cells to silence the nascent immune response. New cancer immunotherapies developed in our laboratory aim to awaken this immune response in order to fight cancer.	
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)?	Data generated in this study will be used to develop the next generation of immunotherapeutic treatments for broad a range of cancer types, to investigate and refine the mechanisms that underpin current immunotherapies and to produce preclinical data to support Phases I-IV clinical trials.	

What species and approximate numbers of animals do you expect to use over what period of time?	We plan to use approximately 20,000 mice over a period of 5 years.
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?	This project has been designed so as to reduce adverse effects of cancer growth on mice to a miminum, with strict limits on the size and progression of tumours grown in mice. Overall, we expect to observe a moderate level of severity in mice used in this study. Rigorous monitoring of adverse effects will be maintained, and any mouse approaching the predetermined humane endpoints will be euthanised via an approved Schedule I method as will all mice that complete their assigned studies with no adverse effects.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In vitro and ex vivo alternatives will be sought to replace any planned work in this study. However, the biological systems investigated in this study are of a complexity much greater than can be accomplished in vitro or ex vivo as a whole. Therefore, the use of animal models is essential to understand the intricate interplay between tumour cells, the tumour microenvironment and the entire immune system.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The size of experimental groups and the repetition of experimental conditions will be kept to a minimum via statistical analysis of preliminary data and previous studies. Recent advances in genomic and cytometric technologies allow us to generate a greater quantity of data from each study, thus reducing that number of studies initially required.
3. 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	This study has been designed to use the simplest and least invasive methods possible to obtain the data required for completion, with the types of tumours grown and the method of tumour implantation implanted to cause as little distress to the mouse as possible. Duration of tumour growth will be kept to a minimum and use of noninvasive imaging techniques will be employed to monitor tumour progression and

costs (harms) to the animals.	adverse effects.

<table>
<tr><td>Project 43</td><td>Preclinical evaluation of cancer therapeutics</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>cancer, chemotherapy, immuno-oncology,</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td><u>No</u></td></tr>
<tr><td>Translational and applied research</td><td><u>Yes</u></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td><u>Yes</u></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td><u>No</u></td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td><u>No</u></td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td><u>No</u></td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td><u>No</u></td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td><u>No</u></td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>

Identify tolerated dose levels of test drugs and side effects not predicted by cell culture based model systems.
Study the effects of the drug on the body, and also the effects of the body on the drug.
Demonstrate that anticancer activity can be shown at specified doses and with dosing schedules that are tolerated.
Identify the best tumour models to use pre-clinically that correspond with a specific therapeutic target.

</td></tr>
<tr><td>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)?</td><td>The aim of the data generated in these studies is to provide pre-clinical supporting information for clinical trial applications. A drug requiring evaluation will be supplied to Epistem along with summary evidence supporting the rationale for testing the agent. By having a much more thorough investigation into the efficacy and mechanism of action of a drug they will be able to make a more informed decisions on whether to proceed into clinical trials, reducing the risk of later stage failures. This will speed up the clinical trials</td></tr>
</table>

	and make them less expensive. The benefit is therefore a reduced number of unproductive human volunteer studies (and a reduced risk of adverse effects) and most importantly the development of improved and more effective therapeutics. The benefit to patients will be the identification of new anti cancer drugs. These studies will help identify the best potential drugs early in the drug development process.
What species and approximate numbers of animals do you expect to use over what period of time?	We would expect to run 150 studies on behalf of sponsors using approximately 7,500 mice and 1,150 rats over the 5 year duration of this project licence.
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?	Side effects of tumour treatment can include lethargy, anaemia, loss of appetite, diarrhoea, dysuria, bruising, bleeding or peripheral neuropathy. Animals exhibiting these signs will be humanely killed. This is likely to manifest as weight loss. A general dose limiting sign will be a 15% loss in bodyweight, and animals showing this will be considered unwell. Any mouse reaching a 20% bodyweight loss, or any rat reaching a 25% bodyweight loss along with other signs of distress will be humanely killed (schedule 1 method). Subcutaneous tumours may grow to a size that could cause discomfort or interfere with the animals' ability to satisfy thirst or hunger. Also, tumours could ulcerate through the skin dependent on the cancer type, or if intra-tumoural therapy is administered. Animals will be killed if their ulcers do not heal within 48 hours or if their tumour reaches more than 15mm in any direction. Injection at the tumour site may cause temporary bleeding which should stop within a few hours of injection. In the unlikely event that bleeding does not cease, and if the animal shows signs of discomfort, it will be humanely killed. Orthotopic (implanted at the natural tumour site, eg a breast cancer cell line injected into the mammary fat pad) tumours may have site specific adverse

	effects and elicit metastatic disease. *Metastatic disease will be monitored by imaging of the whole body wherever possible.* However if such techniques cannot be employed using a specific model, *any deviations in physiology or behaviour will be treated as indicative of metastatic disease, and animals will be humanely killed when there is loss of condition consistent with the severity limit as defined by the Home Office regulation.* For leukaemias, animals may gradually become weak, lethargic and lose body weight. Infiltration of the spleen or liver can lead to enlargement of these organs which may be palpable. Any animals showing signs of distress or symptoms at the limit of moderate severity will be humanely killed. Immunocompromised mice will be maintained in Individually Ventilated Cages in a barrier environment to avoid unwanted infections. If animals develop unwanted infections or surgical wound complications, they will be given antibiotic treatment after advice is sought or will be humanely killed. Animals will receive analgesia following surgical procedures such as bone marrow aspiration or orthotopic tumour implantation. Prolonged periods of anaesthesia can lead to animals losing body temperature. To counteract this, animals will be warmed throughout the procedure, either by the use of heating mats, warm air blowers or temperature regulated stages. . All work will comply with the UKCCCR (United Kingdom Co-ordinating committee on Cancer Research) guidelines for the welfare of animals in experimental procedures.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The programme requires that the models used are ones which closely mirror human disease. All compounds to be tested would have previously been screened in relevant *in vitro* models to determine those candidates suitable for *in vivo* testing. Rodents (rats and predominantly mice)

	are suitable for these studies as the work cannot be conducted in lower vertebrates, invertebrates or cell lines due to the poor resemblance of these options to the clinical setting. Animal models address issues which current in vitro tests cannot accurately determine.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal models will be restricted to the minimum number of animals needed for a statistically valid result. The number of animals used will be the minimum safely necessary to allow meaningful statistical analysis of the data generated. The most important aspect of the proposed programme of work that will reduce the number of animals used is careful selection of drugs, on the basis of preclinical data. Only those potential drugs that offer a realistic prospect of therapeutic exploitation will be investigated. The investment by the team in the purchase of small animal imaging technology also reduces animal numbers in these experiments. The development of disease can be followed in each animal over time, abrogating the need to humanely kill satellite groups to examine disease progress, and thereby reducing total animal numbers.
3. 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.	Rodents provide a cost and time effective platform in general for most pre-clinical testing. For the purposes of oncology testing, the use of higher species is not required because there is a wealth of knowledge on different types of cancer in rodents, as well as decades of in-house expertise with such models. Internal expertise, and more recent technological advances, such as the use of whole body imaging, allows for a more refined study design that will minimise the number of animals. These techniques will maximise the output and will provide a more thorough assessment. They will also help in selecting the best models.

Project 44	**Personalised Tumour Graft**	
Key Words (max. 5 words)	Tumour, Human, Personalised	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	✓	Translational and applied research
	✓	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This is a service licence for producing a patient derived xenograft tumor model in mice for the purpose of predicting the best clinical treatment for the individual.	
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)?	Generation of personalized tumorgrafts from cancer patients allows extensive personalized drug screening studies leading to the selection of potentially clinically effective agents. This predictive pre-clinical platform and tumour specific data informs physicians for personalized patient care.	
What species and approximate numbers of animals do you expect to use over what period of time?	Approx 1500 mice over 5 years	
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	Expected severity MODERATE Immunocompromised mice will be used for subcutaneous implantation of patient derived tumour material performed under general anaesthesia.	

happen to the animals at the end?	These animals will be maintained in barrier conditions to allow tumour growth. To optimise tumour growth implantation media will be used to coat the tumour prior to implantation – this is tolerated well by the mice and improves engraftment rate. For hormone dependant tumours (eg breast cancer) subcutaneous slow release oestrogen pellets may be implanted to improve tumor growth. The oestrogen levels are optimal to encourage growth but negate any adverse effects in the mouse. Expected adverse effects due to tumour burden are minimal, this is closely monitored by palpation and clear endpoints are established in line with NCI guidelines. Implanted animals that fulfil strict animal health and welfare criteria will be shipped to a laboratory in the USA for drug testing. Any animal that does not meet the strict conditions for shipment will be killed humanely.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It has been reported in the literature that traditional Cancer cell- lines (in vitro and in vivo) cannot be used to predict drug activity in the patient. There are no other suitable non-animal alternatives for predictive drug testing at this time.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals used for the engraftment is typically 3-5 per case and depends on the amount of patient derived tumour material that is available. Maintenance Maintenance of stocks of immunocompromised mice are kept to a minimum and closely monitored to minimise wastage of animals that become too old for the xenograft procedure.
3. Refinement Explain the choice of species	Close monitoring of tumour growth will be performed by experienced technicians.

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.	Only animals with a low tumour burden that will not be adversely affected during transit are shipped to the drug testing laboratory.

Project 45	**Assessment of Novel Cancer Therapeutics**	
Key Words (max. 5 words)	Tolerability, Pharmacokinetics, Cancer, Mouse, Rat	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall aims of this project licence are to provide data relating to the metabolism of cancer therapies in the mouse or rat and to determine the animal's tolerance of novel cancer therapies. These data will help determine suitable candidates for further testing in vivo. Compounds that have suitable levels in the blood and are well tolerated in mice or rats may be used subsequently in larger more complex tumour bearing studies where tumour size (efficacy) and relevant tissue analysis (pharmacodynamics) are performed under a separate previously authorised Home Office Project Licence. This licence is not intended to determine the limit of tolerability, all therapies administered under this authority will have been tested in vitro for potency and modelled for suitable properties at a predicted efficacious dose. The tolerability of therapies will be defined as the	

	completion of the study as per protocol without the need for intervention due to a combination of any 2 clinical signs such as, weight loss, abnormal animal behaviour (separation from cage mates etc), ruffled fur, hunched posture and additional clinical signs typically representative of poor health in mice and rats. Global estimates predict a substantive increase to 19.3 million new cancer cases per year by 2025, due to growth and ageing of the global population. Whilst current cancer treatments provide some survival benefits (50% survive cancer for 10 years or more in 2010- 2011), they are often associated with significant side effects. Thus there is a clear need for improved and better tolerated medicines that can be used either alone or in combination with existing or other new therapies.
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)?	The benefit of this project will ultimately be the introduction of new and improved therapies for the management of cancer. This project licence will enable therapies to be identified as suitable in vivo candidates for progression into disease and/or surrogate models of cancer. Compounds/substances that are tested in this project licence will be identified as suitable/unsuitable for further testing in larger more complex models of cancer in both mice and rats.
What species and approximate numbers of animals do you expect to use over what period of time?	Only rats and mice will be used on this project. Approximately 5000 mice and approximately 500 rats will be used over a 5 year period. The total number of animals will be approximately 90 per Month.
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?	As these compounds/substances are novel or combinations of novel and standard therapies there is a possibility they will cause adverse effects such as weight loss, ruffled fur and changes in typical social behaviour. Animals will be closely monitored at least twice daily for signs of any adverse effects and humanely killed if these persist.

	There may be adverse effects related to compound administration, recovery anaesthetic and blood sampling. These will be minimised by ensuring all work is performed and animal care and welfare is assessed by highly trained Home Office Personal Licence holders and animal care staff. All experiments will be designed to include the minimum number of procedures performed on the animals as possible. The protocol in this licence is classified as moderate severity with over half of studies anticipated, as being in the mild severity classification. Animals will be culled at the end of the study via a humane method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Non-animal alternatives are used in the identification and selection of compounds and generally include measurements of the likely effect of the agent on the target cells. Cell based assays not involving live animals to predict exposure in vivo are used before selection of compound substance for use under this project licence. Only compounds/substances predicted to have good exposure will be selected for studies in vivo. Given the complexity of the mammalian system and the factors such as bloavailability and metabolism the whole animal is needed for the studies proposed in this licence.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To maximise the scientific integrity of data generated and to use the minimum number of animals possible, statistical expertise will be applied to all experimental design and analyses. Refined techniques of blood sample analysis allows for fewer animals to be used as less blood volume is required for each sample.
3. Refinement	The overall plan of the work is to support the discovery and development of novel cancer therapies to benefit

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.	human health in the treatment of cancer. Only rats and mice including immune-deficient strains are used on this licence. Using non-mammalian species of lower neurophysiological sensitivity is not possible since they lack appropriate tissue physiology. Although exact replication of all pharmacokinetic parameters between species is not possible, many features of human Pharmacokinetics can be predicted from those observed in small mammalian species unlike effects seen in lower organisms. The most appropriate species and strain of mice and/or rats will be chosen based on previous data that has been used to generate single agent efficacy data. Mice will be used in the majority of studies unless there is a scientifically relevant reason that mice cannot be used, for example, compound metabolism issue with the compound. The choice of strain will be driven by the choice of tumour models used in separate Project Licences. For human tumour lines immune-deficient animals are required to support the growth of the tumour, the least immune-deficient strain required to promote good, reproducible tumour growth will be used. For anaesthetic protocols the optimal anaesthetic regime relevant for the species/strain will be developed and used. Pain relief is not anticipated to be required. If necessary, pain relief will be used with the most appropriate and effective treatment available.

Project 46	**Enhancement of targeted radiotherapy of cancer**	
Key Words (max. 5 words)	targeted radiotherapy	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our goal is to optimize the effectiveness of radiotherapy delivered in the form of radioactive drugs. We aim to achieve this by amalgamation with drugs, called radiosensitisers, that interfere with the defences of tumour cells which counteract radiation-induced damage.	
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)?	This programme of work will be carried out in parallel with ongoing clinical studies. Through our alliance with oncologists involved in the management of patients with neuroblastoma and prostatic carcinoma, the outcome of these studies will be the rapid translation of positive laboratory findings into improved treatment for the benefit of cancer patients.	
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 550 laboratory mice, lacking a thymus gland hence incapable of rejection of transplanted human tumour cells, will be studied per year in order to determine the time-dependent distribution of radioactive drugs in tumour and normal organs and the inhibition of tumour growth after the administration of	

	various schedules of combinations of. radioactive drugs and radiosensitisers.
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?	All procedures have moderate severity limits. Failure to recover from anaesthesia is rare (<1% as indicated in our previous studies). We have observed no adverse effect from the injection of radioactive drugs. Likewise, these treatments have induced no sign of marrow toxicity, such as anaemia. Intravenous injection carries the risk of tail vein damage and subcutaneous injection can result in transient discomfort. Intraperitoneal injection carries the risk of organ puncture. Possible adverse effects of giving drugs by mouth are damage to the gullet and inadvertent dosing into the windpipe. At the end of experimental studies, mice will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Knowledge of the time-dependent distribution of drugs in tumour and normal organs can only be obtained using animals. They are also necessary for the assessment of the impact of the various therapeutic combinations on tumour and susceptible normal organs, especially bone marrow. Furthermore, the radiation dose delivered to tumour, whole body and various normal tissues cannot be determined from the study of tumour cells in culture. Instead, this important information can be gained only by experiments involving live animals. We have developed alternative models, based upon multicellular spheroids (aggregates of tumour cells), for initial assessment of new treatments before embarking on animal experiments. Accordingly we are able to minimise the number of animals involved in refining radioactive drug therapy.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The extent of animal studies will be reduced through the following procedures: - Evaluation in animals will by applied only to those schemes which show merit in tumour cells grown in the

	laboratory. - Investigations using cultured cells will indicate the best sequencing of treatments. - Exploitation of the tumour spheroid model will enable the preliminary study of the effectiveness of radioactive drug treatment. - The numbers of mice employed will be advised by a consultant statistician. This number will be the minimum required to provide confidence in experimental results.
3. 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 nude mouse is the simplest mammal that can be used for the intended studies of radioactive drug treatment of cancer. It enables the growth of tumours under the skin and is widely recognised as providing useful information in cancer research. This makes possible the comparison of experimental results between research groups. Targeted radioactive drug treatment requires the possession by the tumour of a target which is bound by the drug. Our animal models satisfy this requirement by virtue of their possession of three different targets. To determine the effect of radioactive drug treatment on parts of the tumour which are not bound by radioactive drug, we will use mosaic tumours, composed of various proportions of targetable regions. In the first instance, the evaluation of new treatments will be carried out using cultured tumour cells and only promising schemes will be carried forward for experimentation in animals. All protocols have moderate severity limits. Procedures will be refined in the light of experience, according to the NC3R guidelines and Guidelines for the welfare and use of animals in cancer research (Workman et al, 2010, Br J Cancer, 102:1555— 1577). Furthermore, animal husbandry will be performed by dedicated, experienced staff.

Project 47	The molecular basis of lymphoma and leukaemia.
Key Words	lymphoma, leukaemia, mouse models
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;

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

Identifying the molecular changes that drive human cancer has proven useful to the development of novel therapies, however it is difficult to determine which mutations are contributing to tumour development since many have no effect on a cell. The study of mouse models of cancer complements the study of human cancer by confirming which mutations are contributing to malignancies.

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)?

Identifying mutations that contribute to cancer has already led to the development of a number of novel drugs for various types of cancer including lung cancer, breast cancer and leukaemia. The goal of our studies is to identify new drug targets and consequently novel drugs that can be used to treat patients with disease that is currently incurable.

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

All our animal studies are performed with mice. Over a 5 year period we expect to use up to 3000 animals for the generation and maintenance of genetically modified mouse strains used in our studies. Some of these animals will then be used in tumorigenesis assays where we study the genes that drive hematopoietic

malignancies and test treatments of these malignancies. Additionally we expect to use up to 2000 non-genetically modified animals in experiments where mice are transplanted with lymphoma cells.

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?

Adverse effects include the symptoms of hematologic malignancies (weight loss, shortness of breath, growth of lymphoid organs) and may be of mild or moderate severity depending on the experiment. These symptoms are monitored by regular checks for symptoms by the investigators and animal caretakers. All animals will be sacrificed at the end of use by a Schedule 1 method. A small number may also be transferred to other protocols that cover the use of genetically modified strains of a similar type and/or the use of similar hematologic malignancies.

Application of the 3Rs

Replacement

The behaviour of blood cells and blood derived cancers is highly dependent upon their interaction with a diversity of cell types in different organs (such as the spleen, lymph nodes, bone marrow and thymus). Whilst some blood cells and lymphoma cell lines can be cultured *in vitro* this does not fully model the behaviour of these cells *in vivo*. Thus work with animals is required to properly study lymphoma and leukaemia.

Reduction

The number of mice to be used is based upon our calculations, previous experience and the literature and will be the minimum numbers of mice required to achieve the objectives of this license and obtain significant and accurate results. Our breeding programme and experimental designs will be streamlined to obtain the maximum amount of data from a single animal.

To reduce experimental variation between animals and ensure scientific rigour.
- mice are maintained on an inbred background and housed in identical conditions.
- mice are randomly assigned to each experimental group and analyses are conducted blind.
- lymphoma symptoms are scored by the same individuals.
- tissue harvesting and analysis is conducted using standardised protocols.
- cells injected into mice are maintained and transduced using identical conditions.

Experiments will be planned so they can be published in accordance with the NC3Rs' ARRIVE guidelines.

In addition where possible we will use cell lines and culture mouse lymphoma cells *ex vivo* to minimise the number of mice we use.

Refinement

We use mice to study hematologic malignancies because they are the lowest mammalian vertebrate species with a hematopoietic organ system that resembles humans. Some of our work requires the study of malignancies *in vivo* in order to include the complex interactions between different blood cell types and other cell types that support blood cell development in the bone marrow, lymph node and spleen. We also study the immune response against lymphoma cells.

Our mouse models use genetic lesions corresponding to the most common mutations found in human hematologic malignancies so that they closely resemble human disease.

To minimise welfare costs in this project, animals will be monitored and detailed records kept tracking the expected latency of each model based on prior experience. Where practical we will design experiments such that animals are sacrificed during a time course prior to the full onset of symptoms.

All animals will be housed in groups where possible, with appropriate environmental enrichment and fed according to current institutional 'best practice'.

Project 48	Pre-clinical assessment of new anticancer agents
Key Words	Oncolytic virus, immune stimulation, vaccine, combination treatment, anti-cancer agent
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;

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

In our laboratory we have developed several new treatments for prostate, pancreas, ovarian and lung cancers. In this project we plan to determine how these novel agents act in live animals (mice) before evaluation in patients. To enable us to determine safety and efficacy we need to study the following:

1. Develop new and realistic models of human cancer in mice.

2. Evaluate the efficacy, safety and mechanisms of actions of our new anti-cancer treatments in the animal models.

3. Examine how our new anti-cancer treatments interact with conventional chemotherapy and radiotherapy.

4. Evaluate how our new anti-cancer treatments interact with the host immune response and how the immune system can be harnessed to improve long-term protection against cancer.

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)?

By developing models in mice of human cancer we will more accurately be able to understand how human cancer progresses and whether our novel therapies are safe and efficacious before evaluation in patients. It will be possible to identify unexpected side effects and unwanted or desired interactions with conventional drugs. The

overall benefits are that novel drugs will be thoroughly tested in live animals before they are administered to patients to ensure safety and potential efficacy.

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

We will use up to 6500 mice that are supplied by authorised breeders during the entire 5-year period. The animals will have either a normal or a defective immune system dependent on the exact study protocol. These strains are well established and produce healthy animals. In some studies we will also use animals that have been genetically modified to develop cancers spontaneously, similar to cancer in patients, such as mice predisposed to pancreatic cancer.

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?

From our past experience we predict that the planned studies will cause only mild or moderate discomfort. We will monitor treated animals daily and do not expect any severe side effects. However, if an animal appears to suffer for example, by loss of weight, not eating or drinking, shivering, not grooming, is lethargic or has a hunched posture, it will be humanely killed using regulated procedures. At the end of each study when all results have been collected, all animals will be discontinued by humane and regulated procedures.

Application of the 3Rs

Replacement

All the new anti-cancer therapies that we plan to test in this project have been, or will have been, extensively tested in the laboratory before we test them in live animals. Our laboratory experiments will identify therapies that are most likely to be successful in patients and will supply us with invaluable information. Only new therapies that demonstrate repeated efficacy in the laboratory will be further examined in live animals. We have already minimized the number of animals that will be needed by developing sophisticated non-animal models and will continue to improve on these models in parallel with this project. However, laboratory-based models cannot predict how the new therapies distribute within an organism or how they affect a living animal. These aspects can only be assessed in realistic models of cancer in whole animals.

Reduction

We aim to use the minimal number of animals necessary to determine safety and efficacy by first examining the new therapies in the optimized laboratory models described above. Furthermore, we will always perform statistical power calculations to determine minimum number of animals required to address the stated primary endpoint of the study in a single experiment. Proper calculation of the required

number of animals will avoid un-necessary repetitions of studies that may be wasteful. Our routine use of imaging of live animals during the study enables the observation of a single animal prospectively throughout an experiment rather than relying on sacrifice of separate groups of animals at each time point.

Refinement

The main refinement is the use of previously established more sophisticated and realistic models of cancer that we will further develop during this project, both laboratory- and animal-based. We have over the years decreased the number of studies that use implantation of human tumour cells under the skin of animals without an immune system. These models are suitable for certain studies but give only basic answers. Therefore, we will use models that more accurately reflect human disease. The most simple of these involve injection of tumour cells into the abdominal cavity that provides an accurate model of human ovarian cancer. We have also developed models where tumour cells are injected directly into the relevant organ, e.g. the pancreas. These tumours develop complex interactions with surrounding normal tissue similar to the situation seen in human cancers, enabling us to determine the efficacy of our new drugs under more relevant conditions. Finally, we will use animals that have been genetically modified so that tumours form spontaneously similar to tumours in humans. These models are the most realistic available and reflect the most up-to-date information about human cancer biology.

Only experiments with mild or moderate severity will be performed. Anaesthetics and pain-relieving agents will also be administered appropriately in order to minimize animal suffering.

Project 49	Analysing cancer: immune cell interactions involved in metastasis
Key Words	cancer, imaging, animal models, immune system, cell signalling
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;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

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

Our objective is to understand the events, cellular and molecular, that contribute to tumour spread through the body, which is the leading cause of deaths of patients with cancer. By applying novel imaging techniques, we aim to understand the interactions between molecules and cells in different animal models of cancer. Our ultimate goal is to define new biomarkers that can be used for diagnostic and for tracking tumour cell progression and development.

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)?

This project will provide important information on how and why cancer cells spread in the body. We will gather information on the role of cells of the immune system in modulating this invasion process. We believe this will be the basis for the development of new strategies to fight cancer and will be of benefit for patients who may be stricken with this disease.

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

Mouse We expect to use an approximate number of 6500 over a period of 5 years.

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?

Immunodeficient and genetically modified mice might be more susceptible to infections as compared to normal mice. Their health will be monitored constantly during our experimental procedures and housed under sterile conditions to limit the potential of infections. Mice that receive tumour cells will be monitored carefully in order to assess tumour growth. Before the tumour reaches a specified size, experiments will be performed and the animals will be killed immediately thereafter. If animals develop any signs of pain or distress they will be humanely killed. In the last 5 years we have refined our experiments to focus upon the early stages of tumour metastasis. Our microscopic imaging is now used at these earlier timepoints so abbrogating the need to image mice at later timepoints when there is a larger primary tumour and large metastatic lesions. Patients who die from cancer often succumb due to growth of metastatic lesions and so understanding the key early steps leading to establishment of metastasis will be crucial in determining novel treatment strategies for patients. In the next 5 years we hope to start looking at treatment strategies and the monitoring the effect of treatments through the use of whole body imaging. The use of established drugs will allow us to refine our treatment protocols according to published literature.

Application of the 3Rs

Replacement

We need to use animals in our studies because different aspects of the tumour environment can not be modelled *in vitro*. These include the presence of a wide range of non-tumour cells, the blood flow and oxygen levels, which can have an important effect on tumour cells behaviour. Tumour cell biology has been extensively studied *in vitro* with often contradictory results resulting from the highly variable and artificial nature of the assays used. In order to develop better *in vitro* models we need to understand how tumour cells behave *in vivo*. The direct analysis of tumour and surrounding cells in living tumours will provide this information. Finally, the development of anti-tumour strategies can only be done *in vivo*.

Reduction

The number of animals in individual study groups will be chosen to ensure a clear answer to the question being asked while using the minimum number of animals. The number of mice per sets of replicate experiments will be estimated based on calculations so the minimum number enough to provide statistical significance will be used. Once the mice are culled, we will dissect and use all the relevant tissues. Whenever possible cell suspensions and tissues will be frozen for future additional analysis.

We are committed to reducing our animal numbers and in the last 5 years we have made significant progress with this through the use of the mammary imaging window technique. This allows longitudinal imaging for a period of up to 2 weeks enabling observation of a tumour development over time and at the same time reducing animal numbers.

In the next 5 years we plan to begin using a relatively novel 3D culture technique to replicate *in vivo* tumours. This technique has been studied using patient-derived tissue and we hope to learn this *in vitro* technique from colleagues within the Breast Cancer Now Unit. Our collaborators at this unit have recently obtained a NC3R project grant to use this technique. We will validate our results from these new 3D culture techniques i*n vivo* to establish that this technique replicates the *in vivo* situation.

Refinement

Mice have been chosen as the experimental animal of choice as they have the lowest neurophysiological sensitivity while still being able to support the growth of tumour cells. The availability of immunodeficient strains, lacking mature B and T cells, permits the growth of tumours. Following tumour induction animals will be monitored up to 3 times per week and the volume of the tumour will be assessed. Tumours will be only allowed to grow to a suitable size for future surgical window chamber implantation or routine passaging in other animals (no more than 5-10% of total body weight, in accordance with current guidelines for the welfare and use of animals in cancer research. For the tumour imaging in live mice all procedures will be performed always under anaesthesia and the animal will not be allowed to regain consciousness before being killed by a schedule 1 method.

In the last 5 years we have refined our experiments to focus upon the early stages of tumour metastasis. Our microscopic imaging is now used at these earlier timepoints so abrogating the need to image mice at later timepoints when there is a larger primary tumour and large metastatic lesions. Patients who die from cancer often succumb due to growth of metastatic lesions and so understanding the key early steps leading to establishment of metastasis will be crucial in determining novel treatment strategies for patients.

In the next 5 years we hope to start looking at treatment strategies and monitoring the effect of treatments through the use of whole body imaging. We have recently began a collaboration to look at the effects of an non-reversible EGFR inhibitor that has been FDA approved for use in non-small cell lung cancer (NSCLC). The recent publication describing this novel inhibitor defines treatment protocols in terms of doses and timepoints and so has refined our experimental protocol. We are particularly interested in using whole body imaging to detect tumoural changes post-treatment that may indicate tumour resistance to treatment.

Project 50	Generating New Mouse Models of Human Cancer
Key Words	Cancer, Model, Transgenic
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;

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

Cancer, a major health issue worldwide, is a multistep disease resulting from a series of genetic mutations in genes referred to as oncogenes and tumour suppressors. Understanding how these genetic lesions change the normal cell to a cancerous one is vital if we are to prevent and treat cancer. Only in the context of the complete living animal can we fully understand how cancers develop, invade and spread to other organs. Using genetically engineered mouse (GEM) models with the same genetic mutations as in the human disease (so called 'patient-like' animal models) we can investigate the biological consequences of these lesions in cancer progression and identify those genetic events and signalling pathways which work together to drive invasion and metastasis. Such information will enable us to design new and targeted therapeutic approaches.

The ultimate aim of the project is to use mouse models of human cancer in fundamental cancer biology research and in identifying new therapeutic targets.

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)?

This project will deepen our understanding of the underlying causes of cancer in general and of specific types of cancer which currently have a poor prognosis such as pancreatic cancer. Knowledge of the genetic causes will dramatically improve our ability to diagnose, treat and prevent cancer which affects one in three of the human population. We will also use mouse models to identify and test new therapies which

will benefit cancer patients. This may involve finding novel ways to treat the disease, for example by altering the way cancer cells utilise nutrients.

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

This project uses mice (including genetically engineered models). We expect to use up to 6,800 mice per year over 5 years. It should be noted that 70% of these will not undergo scientific procedures, but will be used solely for breeding and maintenance of colonies. Breeding GA animals naturally results in the production of wild-type and other animals which do not carry the correct set of genes. Consequently these animals cannot be used in procedures or for further breeding.

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?

Approximately 70% of the mice will not show any adverse effects relating to their breeding and not undergo any procedures except for ear notching for identification and genetic testing. These will be humanely killed when they are no longer required for breeding. Some animals (around 20%, up to approximately 1500 animals each year) will receive hormone injections. This will result in no more than transient discomfort and no lasting harm. A smaller number of animals (<10%, up to approximately 600 animals each year) will undergo surgery for the implantation of fertilised oocytes, generated by in vitro fertilisation or other similar techniques. These animals receive anaesthetic during the surgery and also peri and post-operative analgesia to minimise discomfort. These animals are closely monitored and expected to recover fully from surgery within 24 hours. At the end of the study all animals will be humanely killed and where appropriate, tissues collected at post-mortem to gather as much information from the study as possible.

Application of the 3Rs

Replacement

Although many aspects of cancer research can be conducted using cells in the lab, it is not easy to fully model the complexities of a tumour which is an interaction of many different cell types (tumour cells, immune cells, blood vessels). Furthermore, the ability to monitor how cancer cells invade and spread to other organs (a process called metastasis) is very difficult to do other than in a mammalian model. Finally we know that cancer cells respond differently in the lab to anti-cancer therapies as they do in the context of the living organism and so testing the efficiency of such therapies requires a live animal.

Reduction

We perform preliminary experiments using only a few animals, before scaling up to the appropriate numbers for a full study. Numbers are calculated based on our

experience using the same models, published literature and advice of our in-house statistical experts. We also share animals between experimental groups where possible - e.g. when we need normal animals for controls, we can often obtain these from our breeding colonies where they would normally not be needed in a study. We constantly optimise our breeding strategies to minimise the number of animals needed to achieve the desired genotypes for our studies To reduce numbers of experiments we also perform studies using cell lines or 3D models so that only our strongest hypotheses are tested in the mouse.

Refinement

We use mouse models with the same genetic changes that are known to cause human cancer – so accurately replicating the human disease. These genetic changes are introduced in a precise and controlled manner, such that the effects can be specifically induced in the tissue of interest so that unrelated effects in other tissues do not occur. All animals are monitored regularly for signs of abnormal behaviour and are humanely killed if they exhibit moderate adverse symptoms. All staff are expertly trained in these clinical signs. Regular monitoring of mouse welfare allows us to complete studies at the earliest endpoint.

Animals are housed in a dedicated facility proactive with environmental enrichment and receive anaesthesia and analgesia as appropriate.

Project 51	SKIN CANCER SURVIVAL IN THE AGEING POPULATION
Key Words	Melanoma, microenvironment, squamous cell carcinoma, ageing
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;

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

Our aim is to improve the prevention, diagnosis, biomarkers of response and ultimately the survival of skin cancer of elderly patients. The use of mouse models enables us to develop new strategies of prevention and care.

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)?

This project will contribute to the understanding of cutaneous and skin cancer biology and metastasis mechanisms specifically in the aged patient. Moreover, we will be able to propose primary and secondary prevention strategies. These animal models will provide more powerful methods to elucidate the underlying mechanisms of skin cancer progression and metastasis and to introduce drugs targeted to the elderly skin cancer patient cohort. Our results will inform adjuvant clinical trails as well as chemical prevention strategies to rationalise management decisions in the clinic based on the in vivo results from our studies. Finally, we expect to publish our work in peer reviewed journals thus sharing our findings with the scientific community.

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

Species: mouse Number of animals: 1000 Period of time: 5 years

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?

The majority of animals are not expected to show signs of adverse effects that impact on their general well-being. Very rarely the severity of these signs may be such that the humane end points may be reached. The majority of the procedures will result in no more than transient discomfort and no lasting harm. All the mice will be humanely culled at the end point of the experiments.

Application of the 3Rs

Replacement

In our lab, we will perform a collection of in vitro assays to understand important points of skin cancer biology. While this approach will and has elucidated many changes experienced by cancer cells, it provides little information about the factors influencing early-stage cancer development in vivo. So far, the majority of our current understanding of carcinogenesis comes from the in vitro analysis of late-stage tumour tissue removed from cancer patients. We will complement these in vitro studies with 3D organotypic skin constructs before we move to in vivo models as well. Importantly, the development of effective cancer preventive/adjuvant therapeutics is an important goal of modern biomedical sciences. To identify potential cancer targets, the processes involved in tumorigenesis must be understood at all levels, which requires the development of model systems accurately mimicking skin cancer progression. Cancer development is dependent not only on the changes occurring within the transformed cells, but also on the interactions of the cells with their microenvironment. Also certain hallmarks of cancer, such as metastasis and angiogenesis, are impossible to study in vitro. Therefore, mouse models are important for studying the in vivo aspects of human cancer development. Wild type and transgenic mouse models can be treated and engineered to develop cancers, which accurately mimic their human counterparts, and have potential applications to test the effectiveness of therapeutics and understand cancer progression and biology. In vitro studies or different in-vivo models such as zebra fish or insects cannot replace the comprehensive view afforded by mice.

Reduction

Our use of in-vitro/3D methods limits the number of animals required for the in-vivo investigation stage. We will also use ShARM, a comprehensive mouse archival resource for the study of ageing related resources. For our transgenic models we will use efficient breeding strategy to minimise the number of mice used to obtain the desired genotype.

The proposed experimental designs and methods of analysis of the results are always in agreement with statistical guidelines and with our bioinformatician scientist to provide meaningful data minimizing the number of animals used in each experiment. The design of individual experiments will generally involve factorial designs, which maximise the information obtained from the minimum resource.

Refinement

Mouse models that we are currently using faithfully recapitulate the human disease. Moreover, the mouse genome shares 98% homology with human genome.

We constantly work to improve husbandry and procedures which minimise actual or potential pain, suffering, distress or lasting harm and/or improve animal welfare in situations where the use of animals is unavoidable. We ensure to provide the appropriate anaesthetic and analgesic regimes as well as appropriate humane methods of culling within animal facility. We use optimal levels of UV light exposure to ensure tolerance whilst achieving our results. We ensure no visualisation of procedures in other animals and transport arrangements between facilities in appropriate containers.

Project 52	Mouse models for tumour stem cells and anti-tumour efficacy studies
Key Words	Cancer, Brain tumours, tumour growth and invasion
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
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

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

Almost all malignant brain tumours represent unmet clinical needs and, on average, the life of a patient with a brain cancer is cut short by 20 years.

Our aim is to find novel brain tumour cancer vulnerabilities and to test novel potential therapeutic agents in a preclinical setting in the context of the complex processes underlying brain tumour growth and invasion.

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)?

Current brain tumour treatments cannot provide a cure and the tumour frequently re-grow despite of DNA-damaging therapy. Therefore, therapeutic approaches that selectively target brain tumour cells more efficiently are urgently needed. However, the complex nature of brain tumours (including invasion of healthy brain tissue, differences between patients and within one and the same tumour) poses a challenge for successful treatment and almost all aggressive brain tumours remain incurable. Our research aims to provide a better understanding of brain tumour biology and we aim to determine the preclinical effects of untested agents or agent combinations (for example chemicals) that could potentially stop the growth of the brain tumour in mice. The testing of agents that reduce tumour growth in animals is required for the ultimate goal of testing a new treatment in the clinic.

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

We expect to use ~800 mice during the next 5 years.

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?

Mice undergoing surgery (intracranial cell implantation) show mild or no symptoms after they awake from anaesthesia. The level of severity is moderate. Intracranial tumour growth may lead to symptoms once the tumours become larger and once mice start showing signs of pain, they will be humanly killed.

Application of the 3Rs

Replacement

Our ultimate goal is to translate some of our research findings, for example the use of a novel anti-brain tumour agent into a clinical test. This route requires pre-clinical studies using animals such as mice providing data that may highlight a so called therapeutic window (meaning that the inhibition of tumour growth outweighs the side effects). The mouse models described in this licence application are required for that purpose because the pharmacological aspects (including blood brain barrier penetration of any given agent) as well as the effects on brain tumour cell biology can be assessed in the relevant environment (brain), hence, allowing for sound analysis. There is currently no replacement for these pre-clinical brain tumour models.

Reduction

All animal treatment experiments that cannot be based on literature (due to addressing a knowledge gap) will be based on comprehensive cell culture analysis including toxicity in cancer cells as compared with non-cancerous (control) cells. Experimental design will be informed by statistical tools that predict variability within the experiment so that the minimum number of animals can be used.

Refinement

Orthotopic tumour models are currently the most refined method to address tumour development in the relevant organ. These cancer models are well established in mice. A publication database search of the key words “orthotopic, cancer, mouse model” retrieved 701 entries as of Nov. 23rd 2010 and 3725 entries as of March 10th 2016. This ~5-fold increase in literature describing orthotopic mouse models during the past 5 years strongly suggests that these models are critically required for (biomedical) research. To study tumour complexity and resistance to treatment it is important to measure tumour behaviour in the mouse model mimicking the situation in patient tumours. Importantly, so called xenograft models reflect hallmark features

of aggressive brain cancer including extensive migration of tumour cells in the brain. These models utilize cells derived from patient tumours and as such reflect well the diversity of tumour profiles observed in different patients. Discomfort and distress of animals will be limited to unavoidable procedures required for the conduct of sound research. We will consider relevant refinement(s) of the surgical procedures and imaging procedures described in this protocol. Intracranial cell transplantations in mice will be performed under anaesthesia and pain relief medication will be given after surgery. Animal will be monitored on a daily basis and will be culled humanly when showing adverse effects.

Project 53	Developing an ocular melanoma model for drug discovery
Key Words	ocular melanoma, uveal melanoma, Patient derived xenograft, MEK
Expected duration of the project	2 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;

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

Uveal melanoma is a rare form of cancer that arises from the eye. Unfortunately it often spreads to the liver and treatments are mostly ineffective. In this project we hope to find out how and why this cancer is resistant to a type of drug called selumetinib. We anticipate that the results of the study will help us design new combinations of drugs (including selumetinib or relted drugs) that will overcome this resistance.

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)?

Uveal (also known as ocular) melanoma is a rare cancer arising in the eye. Unfortunately it spreads to the liver in about half of all cases and is invariably fatal. At present there are no established therapies for uveal melanoma, and resistance to drug therapy is very common. We intend to decipher the main mechanisms by which this occurs thus developing new combinations, which may help control the disease and prolong survival.

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

We will use approximately 300 mice over 3 years.

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?

The severity limit of the experiments is moderate, and most animals will have limited adverse events. All animals will have tumour cells implanted under the skin and these will be allowed to grow up to about the size of a pea. Some animals will have drugs given to them which may cause some adverse effects, however in all cases the side effects will have been established previously and a dose used to minimise these. At the end of the experiment the animals will be killed humanely and tissue extracted for experiments.

Application of the 3Rs

Replacement

Wherever possible we will perform experiments in cell lines or directly on human samples. However, these experiments cannot model many of the effects of growing tumours inside humans such as the presence of other cells, growth of blood vessels into the tumour and varying concentrations of oxygen and nutrients in differing parts of the tumour.

Reduction

We will only use enough animals to establish our model systems and for the purposes of our assays. Where we perform experiments to contrast different treatments, we will perform calculations to identify the minimum number of mice needed to show a meaningful result.

Refinement

While simpler organisms may be used to perform experiments on the basic biology of cells, the experiments we will be performing need us to be able to grow tumours derived from humans. Mice are the simplest model system, which will allow us to do so and then treat with drugs. We will monitor the condition of mice daily and weigh at least weekly. Where animals appear sick we will observe more closely, and if they are not recovering, the animals will be euthanized. Tumours will not be allowed to grow beyond a specific limit which has been established in previous experiments. Where drugs are given, these will be used at a dose that has been shown to be tolerated well by animals without significant adverse effects.

Project 54	Adoptive T Cell Therapy for cancer
Key Words	Cancer, T lymphocytes, T-cell receptor, immunotherapy, adoptive therapy
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;

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

At least one third of the British population will develop cancer and 1 in 4 of them will die from it. Thus, there exists an urgent need to develop more effective anti-cancer therapies. The immune system has the potential to selectively destroy cancer cells and over the last 10 years studies, initially in animals and subsequently confirmed in humans, have shown that immune cells can be used to treat a few types of cancer. This has encouraged further work to improve these approaches, for example, to expand their use to treat many more cancer types. However, this requires several hurdles to be overcome. These include (i) generating immune cells that are able to recognise and destroy the different cancers, (ii) ensuring, following injection, that they reach the tumour site, (iii) ensuring that the tumour cells are not able to evade the immune cells and finally (iv) ensuring that any new approaches are safe

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)?

This project aims to validate novel immune-based therapies designed in our laboratory to target the tumour cells or the blood supply that serves them. The study will use mouse models of cancer and of new blood vessel formation. These involve injecting tumour cells or implanting small pieces of sponge under the skin (into which new blood vessels, resembling those in tumour tissue, will grow). Immune cells are then injected to see if they can prevent tumour growth and/or recognise the new blood vessels. This project will advance our understanding of immune-based

therapies for cancer. It will also help to validate much needed new treatments for cancer which can then be trialled in cancer patients.

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

This project will only use mice bred for research purposes. Mice are the most suitable animal in which to study immune responses to cancer because we understand the immune response in this species more than any other animal and this response is sufficiently similar to that in man to justify use in this way. Furthermore, specialised strains of mice are available that enable us to perform well controlled experiments that will yield high quality information. Some strains also allow us to use human cells in these models so we can study the response of human T cells to human cancer cells. We estimate that we will use 912 mice per year. Where necessary we will initially use pilot studies with small numbers of mice to help determine the minimal number of mice required for an experiment to reliably answer the question being addressed. Also, where possible experiments will be designed to derive the maximum amount of information from the minimum number of animals

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?

In this project some mice will be injected with tumour cells or have small sponges implanted into the skin. These procedures will be conducted under general anaesthesia. The mice will then be injected with immune cells and other therapeutic agents as well as agents that enable us to see the growth of the tumour cells. Occasional blood sampling using a needle will also be required. These procedures will induce some stress due to restraint and transient discomfort from needle insertion. Injecting human T-cells into mice may result in the immune cells attacking the host tissues and the growth of cancer cells could eventually prove fatal to the mice. Some mice will also be exposed to limited doses of radiation to partially deplete their own immune cells and thereby allow us to efficiently introduce “tumour-targeted” immune cells. High doses of radiation can cause sickness in mice therefore we will use doses that are well tolerated. However, any animal showing signs of distress/pain reaching a moderate severity limit will be culled to avoid further suffering. Furthermore, any mice undergoing surgical techniques will be monitored carefully and will receive drugs for pain relief.

Application of the 3Rs

Replacement

All potential immune-based therapies to be tested in mice as part of this project will firstly have been tested in vitro (i.e. without animals) to demonstrate that they look promising. However, these in vitro assays cannot adequately reproduce the challenges we face in treating cancer in a patient. For example they do not replicate

the 3-dimensional architecture of tumours, through which T-cells must spread. They also fail to reproduce conditions for T-cells circulating in the blood stream to stop and move into the tumour tissue. When using T-cells to target blood vessels in tumour tissue, animals are again required to reproduce the defective nature of such vessels, including their unusual blood flow properties. Animal studies can also identify potential side effects of these treatments where the immune response reacts to normal tissues. Finally, the complex interactions that occur between components of the immune response and other tissues cannot be adequately modelled without using animals

Reduction

When designing experiments we will perform statistical analysis to ensure we use the minimum number of mice per group that will be informative. Therefore where necessary initial pilot experiments will be conducted to aid these calculations. Modern imaging techniques enable analysis of cell growth and distribution within the same animal over time allowing us to collect more data from each animal, and thus reducing numbers of mice required for our experiments

Refinement

Mice are the most appropriate species for the work proposed here because inbred strains are available that permit studies of immune responses to tumours. Particular strains of mice are also available that closely model human cancers. Furthermore, the murine immune system is very similar to humans. Mice are considered to have the lowest level of neurophysiological sensitivity among potentially suitable species.

The methods used are designed to involve the least suffering by limiting the number of procedures involved to that required for generating a reliable answer. Furthermore, animals will be anaesthetised before injecting tumour or implanting sponges into the skin and the needle sizes used will be kept to a minimum. Finally, mice will be monitored daily if close to maximal tumour size or when using rapidly growing tumours with risk of ulceration. The advice of the named veterinary surgeon and named animal care and welfare officers will be taken to ensure animal suffering is minimised where possible

Project 55	Drug evaluation in pre-clinical oncology models
Key Words	Cancer, pre-clinical, efficacy, models, Imaging
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;

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

The primary objectives of this project are:

(i) the use of established and validated mouse models of cancer to evaluate candidate anti-cancer agents and combination therapies, to support progression of effective anti-cancer treatments to human trials, ultimately resulting in validated effective final products.

(ii) To support objective (i) through the development of patient relevant pre-clinical models for the evaluation of candidate anti-cancer agents and combination therapies.

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)?

According to studies published in the public domain, 77% of 800 cancer drugs entering early clinical trials failed to reach patients and this failure was attributed in the majority of cases to poor response to the anti-cancer agents in the patients tested. This data highlights the growing need by the Pharmaceutical, Biotech Industry and academia for more patient-relevant and predictive cancer modelling before clinical trials begin especially with new generation of targeted cancer drugs, immuno-therapeutics and combination treatments that are presenting new opportunities for patients with cancer. The aims of this project is to provide the scientific community with a high level of centralised expertise in terms of available clinically relevant cancer models, knowledge and technical capability to improve

decision making on which agents should progress to the clinic and which patients will benefit from the treatment. In some cases this may result in programme cancellations; whilst this may seem of negative benefit, cancellation of candidate anticancer agents either ineffective or unsuitable for further development can be considered a positive benefit in the longer term as it limits the progression of ineffective therapies brought to early phase clinical trials and allows the direction of resources and patients to other projects. As the understanding around the mechanisms behind cancer progression continues to increase, so does the requirement to develop and validate relevant models in parallel to test new strategies. Thus the best way to benefit the scientific institutes that we work with, industry and thus patients as a whole, is by the development of pre-clinical cancer models that exhibit greater patient relevance for their application to the development and testing of novel anticancer agents. We are very proactive in attendance at relevant national and international scientific conferences and actively share our research where possible with the global scientific community through abstract submission to national and international conferences. Once validated, all models are added to the proprietary databases; access to which is free to all users, so one of the immediate benefits of the model development process is that model data, including growth and response to standard therapies, histologic and genetic characterisation is freely available to the scientific community which makes these databases extremely powerful tools for research. What types and approximate numbers of animals do you expect to use and over what period of time? Mice will be used for the entirety of the project as they are the lowest species of animal that allow the modelling of human cancer, and offer the opportunity for genetic manipulation to generate specific models relevant to human cancer. Substantial numbers of cancer relevant models are already validated in-house (100+) making this species most amenable to this course of research to investigate different cancer types which include breast, prostate, lung, brain, bladder, leukaemia, lymphoma, multiple myeloma, colorectal, fibrosarcoma, gastric, head & neck, kidney, liver, thyroid, melanoma, oesophageal carcinoma, ovarian and pancreatic. Over the course of this project we'd expect to use 115,700 animals to model these cancer types, the different stages of cancer and novel anti-cancer agents and combinations.

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

Mice will be used for the entirety of the project as they are the lowest species of animal that allow the modelling of human cancer, and offer the opportunity for genetic manipulation to generate specific models relevant to human cancer. Substantial numbers of cancer relevant models are already validated in-house (100+) making this species most amenable to this course of research to investigate different cancer types which include breast, prostate, lung, brain, bladder, leukaemia, lymphoma, multiple myeloma, colorectal, fibrosarcoma, gastric, head & neck, kidney, liver, thyroid, melanoma, oesophageal carcinoma, ovarian and pancreatic. Over the

course of this project we'd expect to use 115,700 animals to model these cancer types, the different stages of cancer and novel anti-cancer agents and combinations.

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?

The mice used in this project will be used to support candidate anticancer agent development through the following stages/types of projects and model development:
I. Pharmacokinetic testing: Mice will be dosed with candidate anticancer agents to determine the fate of a chemical from the moment that it is administered up to the point at which it is completely eliminated from the body. This information is used to guide dosing regimens to ensure sufficient agent is delivered for a sufficient period of time to achieve effective target efficacy (achieve mechanism of action) in later project stages.

II. Pharmacodynamic (PD) testing: Mice will be dosed with candidate anticancer agents to generate information about the efficacy of the candidate anticancer agent against its tumour target. Taken in consideration with PK analysis they can be used to assess suitability for progression to efficacy testing. The majority of mice will undergo subcutaneous tumour implantation which are visible and measured by callipers (length and width); less commonly surgical tumour implantation into the brain or organs such as liver/lung under anaesthesia which are then measured once/twice weekly throughout the study by imaging under anaesthesia to determine internal size. Once small tumours have established single doses of candidate anticancer agents by standard routes (oral, intravenous, subcutaneous etc.) will be administered followed by scheduled in life and terminal sampling. Tumour and tissue samples will be used to determine impact of agent/dose on modulation of tumour target. The short study/dosing duration and small tumour burden means that the prevalence of treatment-related adverse effects is uncommon in these studies, and any adverse clinical signs are expected to be transient. All mice will be killed at the end of the studies.

III. Tolerability testing: The key aim is to ensure that candidate anti-cancer agents are tolerated at the proposed dose levels/regimens prior to entering into larger efficacy testing protocols. Mice will undergo minimally invasive procedures: short dosing phases (up to 2 weeks) at regimens reflective of follow-on efficacy studies by standard routes (oral, intravenous, subcutaneous etc.); occasionally in-life blood (tail or saphenous vein) sampling or terminal sampling is carried out. Care is made to select a dose regimen to minimise toxicity and informed by PK/PD studies; however, body weight loss (BWL) and/or adverse clinical signs may be evidenced as a result of acute or cumulative dosing. Body weight will be monitored daily and will be used to guide to intervention. Persistent adverse clinical signs e.g. subdued behaviour patterns even when provoked etc. will result in humane killing regardless of body weight measures. If the initial dosing regimen produces evident toxicity, doses will be

reduced by a stepped approach (~30-50%) prior to testing in further tolerability studies. All mice will be killed at the end of the studies.

IV. Subcutaneous (s.c.) efficacy testing: For s.c. efficacy testing, the key aim is to assess the efficacy of candidate anti-cancer agents, either as monotherapy or in combination with other candidate anti-cancer agents on the growth of mouse or human tumours. Mice will undergo subcutaneous tumour implantation by cancer cell injection or tissue implantation under anaesthesia. Dosing of candidate anticancer agents (refined through earlier work) by standard routes will be administered until scientific endpoints i.e. the statistical comparison of treatment to control response or humane endpoints for tumour size, mean diameter ≤15mm, are achieved. Provision of supporting tolerability data or acute phase tolerability studies (section E) means that the frequency of treatment-related adverse effects are uncommon in these studies; however, body weight will be monitored daily during dosing phases and will be used to guide to intervention. Persistent adverse clinical signs e.g. subdued behaviour patterns even when provoked, will result in human killing regardless of body weight measures. All mice will be humanely killed at the end of the studies.

V. Efficacy studies with genetically modified mice which carry the same mutation to that in human colon cancer resulting in similar tumour formation and progression will be dosed with candidate anticancer agents, refined through earlier work, by standard routes until scientific endpoints are achieved i.e. development of adenomas in the small and large intestines by 18 weeks; Alternatively, a surrogate survival format may be employed using a humane endpoint i.e. the onset of anaemia; in this setting, the study can be terminated at that point at which a statistically significant effect on surrogate survival can be determined. All mice will be humanely killed at the end of the studies.

VI. Translational studies: experimental metastasis: Experimental metastasis models mimic latter stages of disease progression that may be difficult to model utilising spontaneous metastasis models where primary tumour size may drive the model endpoint. Mice will undergo tumour implantation by cancer cell injection (intraperitoneal, intracardiac, or intravenous). Dosing of candidate anticancer agents by standard routes will be administered until scientific endpoints i.e. the statistical comparison of treatment to control response (as assessed by optical imaging) or humane endpoints for tumour progression e.g. abdominal distension (peritoneal ascites), changes to gait (bone metastasis), or respiratory changes (lung metastasis.) Provision of supporting tolerability data or acute phase tolerability studies means that the frequency of treatment-related adverse effects are uncommon in these studies; however, body weight will be monitored daily during dosing phases and will be used to guide to intervention. Persistent adverse clinical signs will result in humane killing regardless of body weight measures. All mice will be humanely killed at the end of the studies.

VII. Translational studies: Models implanted in relevant organ sites are known to better model cancer in patients with respect to various criteria as they form a single focal disease area as in the patient situation, facilitate metastatic spread via lymph nodes and show a reduced response to chemotherapy. Mice will undergo tumour implantation by cancer cell injection and surgical tumour implantation into the brain, lung or liver under anaesthesia. Dosing of candidate anticancer agents by standard routes will be administered until scientific endpoints i.e. the statistical comparison of treatment to control response (as assessed by optical imaging) or humane endpoints for tumour progression e.g. abdominal distension (peritoneal ascites), lack of coordination, head-tilt (brain tumour), or respiratory changes (lung tumour) Provision of supporting tolerability data or acute phase tolerability studies means that the frequency of treatment-related adverse effects are uncommon in these studies; however, body weight will be monitored daily during dosing phases and will be used to guide to intervention. Persistent adverse clinical signs will result in humane killing regardless of body weight measures. All mice will be killed at the end of the studies.

Application of the 3Rs

Replacement

In vitro methodologies have replaced animal use in early development phases, particularly in the development of screening assays to refine compound selection, target identification, off-target toxicity or toxicity versus normal tissue cell lines, and can certainly guide and refine the steps prior to moving into in vivo, and minimise subsequent use. However, there is still a requirement to use animals for this project as in vitro assays still do not optimally mimic all interactions between cells and tissues in vivo, such as blood vessel formation, spread to other organs and thereby relevant drug access or the many homeostatic mechanisms in play in an in vivo environment that allows relevant tumour biology drug evaluation.

Reduction

The use of in vitro studies can be used to identify lead compounds, evaluate dose ranges confirming target modulation/expression and relative off-target toxicity which can be used to inform on relevant doses for use in PK, PD and pilot toxicity studies. The use of complex 3D in vitro assays can be applied to pre-screen studies and compound selection prior to advancement into animal testing (thus reducing animal use). Careful use of pilot studies and statistically powering the study design can be used to optimise animal model use and reduce overall use of animals. The use of optical imaging technologies can reduce the number of animals required to generate study outcomes.

Refinement

Mice are the lowest species in which the knock out of the immune system allows growth of human tumours. Mice with a fully functioning immune system also provide

the opportunity to investigate a large panel of mouse cancer models to enable the immune system interplay with the tumour to be investigated. Genetically engineered mice with mutations representing those seen in humans, for example in colon cancer, will also be used to assess the importance of potential oncogenes and mice are the lowest species in which this technology can be applied as require an appropriate mammalian architecture. There will be 2 main approaches to tumour implantation. The majority of mice will have tumours implanted subcutaneously as this enables immediate and accessible measurement of tumour growth for a wide range of cancer models. The second approach is to implant tumour at the site of origin which are more relevant to patients but are more complex and require imaging to track the growth inside the mouse.

Although subcutaneous cell line models lack direct translation to human studies, they are extremely well characterised within the scientific community (peer-reviewed scientific literature). As such they can be a useful tool if used with acknowledgment of their limitations i.e. as a tool to help 'dissect' a specific molecular pathway, gene fusion or driver mutation. In this context they allow a flow of work from early *in vitro* studies, through to PK/PD and efficacy assessments, thus assessing proof-of-concept in a minimally invasive scenario. Furthermore, using optical imaging technology they can be translated to more complex organ-specific and metastatic modelling to offer a more translational context.

PDX models offer significant translational power, they preserve both the genomic integrity and heterogeneity of the original disease and allow the generation of data that closely resemble clinical data. The translational power of PDX models is increased with their application to pre-clinical Phase II-like mouse clinical trials (MCTs) that closely reflect the human trial design, studies can be used to inform on patient selection or dosing strategies in human trials.

Organ-specific models are known to better model cancer in patients as tumour grows in the correct environment which facilitates spread to other organs via the lymph nodes as seen in the clinic and also show a reduced response to chemotherapy. The use of optical imaging will be used to refine the methods used as well as minimise animal suffering, as it allows the opportunity for the determination of a statistically significant result ahead of a scheduled termination, thus potentially reducing the duration of regulated procedures.

Experimental metastasis models mimic latter stages of disease progression e.g. escape from primary site, establishment at the metastatic site, compartmental separation etc. that may be difficult to model utilising spontaneous metastasis models where primary tumour size may drive the model endpoint. Experimental metastasis models are therefore useful for assessing candidate anti-cancer agents directly targeting the development of metastasis, or metastatic treatment strategies which often differ to those used for primary disease in the clinic. In the case of intracardiac administration of cells (i.e. experimental bone metastasis), this results in

a much more refined model than direct injections into the bone as the circulating cells encounter the target organ e.g. the capillary beds of the bones, in the same way as circulating metastatic tumour cells arising from a primary tumour. In the capillary beds they are compelled to invade into the tissue, thus only the clone of the cell population having the required capabilities e.g. tropism conferred by possession of the bone metastasis gene expression signature will survive and grow into a tumour. Direct injection into the bone introduces the cells directly into the site and does not model the escape of cells into the bone site. Furthermore, direct injection may result in the mechanical disruption of the bone itself, which is not only aversive to the animal, but could also compromise the development of lytic lesions that are characteristic of many breast and prostate bone metastases.

The development of relevant pre-clinical models of oncology is a key stage for the evaluation candidate anti-cancer agents and proposals for model development will undergo a review by the company's internal research and development (R&D) committee. Following completion of the model development phase, a report will be generated and the outcomes of the model development process will be carefully reviewed by the R&D committee before the model is considered suitable for use in client studies. As part of the ongoing commitment to the highest levels of scientific output and welfare, a regular review period will be set up for each model following completion of the model development process. This will look to follow-up on the current use and applications of the model to ensure that the most refined science and animal welfare is being utilised. Where areas of potential refinement are identified, these will be assessed through further pilot and validation ?studies.

in summary the methods that minimise animal suffering include the following:

- Pilot studies for the establishment of new tumour lines and refinements to surgical techniques will be carried out on an ongoing basis under the advice of the NVS/NACWO will be sought in this respect.
- All surgical procedures will be conducted in line with established welfare guidelines on aseptic surgery using suitable anaesthesia along with peri and post-operative analgesia.
- Presentation of adverse clinical signs, behaviour patterns or BWL relating to treatment or model progression should be de-risked by supporting work, and managed as detailed in the relevant project plan and protocol sections.
- Sampling will be in line with established welfare guidelines (see general project plan comments), and micro-sampling regimens will be utilised where study design supports this.
- The frequency of dosing will be such that animals fully recover between injections and will not suffer more than transient pain and distress and no lasting harm and there will be no cumulative effect from repeated injections.
- The use of supplemented diet or drinking water may be used for both candidate anti-cancer agents as well hormone supplementation, but in such circumstances, care should be taken to carefully monitor intake, to ensure that that the change in composition doesn't affect normal feeding/drinking behaviour.

- For hormone dependent models (some oestrogen-dependent breast/ovarian models, and some androgen-dependent prostate models) hormone supplementation using the most refined method that results in consistent tumour growth.
- For test agents whose efficacy may be impaired by the blood brain barrier, small proof-of-concept pilot studies may be carried out whereby dosing is achieved by administration directly into the brain or tumour site. Where multiple doses are required use of an intracerebral/intraventricular cannula will be used to reduce the number of invasive procedures.
- Use of pilot tolerability studies to ensure there are no unexpected adverse effects associated with new models or unexpected toxicity as a result of tumour:drug interactions and to ensure the drug levels used are not associated with any cumulative effects.
- Mouse tumours implanted in mice with a fully functional immune system display higher levels of ulceration, therefore appropriate scoring system with defined endpoints and escalated actions has been put in place as a refinement to these models, and ensures that the welfare of the animals isn't compromised and the risk of harm is minimised throughout the model but scientific endpoints are still achieved.
- Non-invasive imaging will be used to refine the methods for all orthotopic and metastatic cell line models as well as minimise animal suffering, as it allows the opportunity for the determination of a statistically significant result ahead of a scheduled termination, thus potentially reducing the duration and of regulated procedures as described in Section D general comments.

- During surgery where there is a need to go through the muscle wall local anaesthesia will be used as additional pain relief. Pain scoring will also be carried for 3 days post op. A standard approach to post-operative pain management is to provide analgesia for 3 days post op, by giving a NVS recommended analgesia in flavoured jelly reducing the need for further procedures, animals are given untreated jelly 5 days prior to surgery to acclimatise. If evidence of persistent pain beyond this time is observed then the animal will be humanely killed. Following surgery the mice will be weighed daily and monitored at least once daily for changes to normal behaviour/clinical signs (typically more frequently) as well as assessment of the surgery site for bleeding.
- All procedures will be carried out in accordance with established welfare guidelines and published scientific guidelines.

Through continual professional development, new techniques for current/new models are developed and refined through the NVS, the research community and animal technology institutions or relevant veterinary expertise.

Project 56	Cellular Immunotherapy of Disease
Key Words	Gene-modified immune cells, tumour immunity
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;
Yes	(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);

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

The overall aim of this project is to test the efficacy and potential side effects of cancer teatments which use immune cells to target and kill cancer cells. This is referred to as cancer immunotherapy. We are most interested in immune cells called lymphocytes (also known as T cells), which can be genetically engineered to kill cancer cells more efficiently.

Our scientific objectives are:

(i) To test T cells modified to simultaneously recognise 2 different target proteins (eg, both on the same cancer cell or one in the cancer cell and one in the affected tissue), by equipping them with new receptors,

(ii) Test additional genetic modifications designed to improve the function of immune cells,

(iii) Generate gene-modified immune cells which are expected to have an improved safety profile, and

(iv) Test combination immunotherapies, ie, use gene-modified immune cells together with antibody therapies.

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)?

Scientific benefits: We expect to demonstrate that immune cells can be genetically engineered to (i) home more rapidly and efficiently to the tumour site/s, (ii) persist for longer in and around the tumour microenvironment, (iii) function more effectively for longer (typically immune cells can get less effective due to exhaustion), and (iv) kill tumour/target cells more efficiently at smaller doses thus minimising the potential side effects and making cell based treatments quicker and simpler to manufacture). Patient benefits: During the term of our last project licence there has been an exponential expansion of early phase clinical activity in cancer immunotherapies, with gene-modified T cells and immune mediated personalised medicine approaches demonstrating efficacy in patients with relapsed and refractory cancers. Our research group has been committed to developing genetically engineered immune cells for clinical use since 2002. In our animal models, the effects of pre-conditioning, context of antigen presentation in vivo, dose of transferred immune cells and likely off-target toxicities have been determined, which have directly informed our current phase I/II trial protocols and formed an essential part of our regulatory submissions. In addition, the immune monitoring assays developed using blood from animals treated with gene-modified immune cells have established a panel of assays, which now form the basis of monitoring assays used in our phase I/II clinical trials. We are now aiming to test 2nd and 3rd generation modifications of the gene constructs where genetic engineering is utilised to enhance the function of anti-cancer immune cells further, both to improve efficacy but also to enhance specificity. In patients, such improvements are expected to increase efficacy of the immune cell therapies thereby increasing disease response rates and/or delaying progression of malignancy. During the course of this PPL we expect to report the outcome of our early phase trials, which are currently recruiting and this will document benefit to patients where achieved.

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

Over a period of 5 years we planned to use approximately 8500 wildtype and transgenic mice.

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?

None of the proposed protocols have expected severity beyond moderate. All protocols or similar protocols have been used before by our research group. In the majority of cases, mice are expected to experience no or minimal discomfort as a result of procedures, and animals are killed, using a humane method, at the end of an experiment to allow full characterisation of their immune systems following the experimental manipulation. Mice used in tumour protection experiments may, however, develop a number of symptoms and adverse clinical signs as a direct result

of tumour growth or from the use of radiation, chemotherapy and/or immune modulating drugs. They may also experience side effects from the immune cells being tested as new treatments for cancer. Common symptoms will include weight gain/loss, loss of appetite, reduced mobility, tumour development (with or without ulceration), ascites and pain or discomfort at the sites of injection or immunisation. In addition, some mice will also undergo repeated general anaesthesia for the purposes of imaging (to assess tumour growth and/or infiltration of the tumours by therapeutic T cells). Mice will be closely monitored for development of such signs and clear endpoints are defined in all protocols in order to minimise discomfort experienced by individual animals. Animals with pain or discomfort will be treated with analgesia and will be closely monitored. It may be necessary to humanely kill mice that do not quickly respond to treatment. Mice with progressive tumour growth will be killed humanely after specific endpoints have been reached, allowing for the isolation of tumour cells and immune cells for ex vivo analysis. All mice will be killed at well specified humane endpoints of at the end of the experiments by a schedule 1 method

Application of the 3Rs

Replacement

In order to fully understand interactions between the immune system and tissues affected by cancer, infection and diseases related to dysregulated immunity, we need to use transgenic/genetically altered mice bearing alterations in immune related or disease-causing genes. The use of in vivo models is essential to refine existing (and develop new) cellular therapies as they allow us to detect in vivo homing and persistence of transferred cells, their efficacy in modifying/preventing disease and off target toxicities.

Reduction

The efficiency of animal usage is maximised in consultation with animal technicians, by careful control of breeding to meet research needs with respect to numbers, phenotypic uniformity and health. We aim to ensure that mouse numbers are kept as small as possible for the duration of the project.

Most of the mouse models and protocols are already well established in our project. As a result, we have already optimised the conditioning regimens and numbers of immune and/or stem cells to be adoptively transferred in order to achieve a therapeutic effect.

The number of animals used per experiment is calculated based on (i) power analyses, generally using a significance level of 5% (80% power) and a least practicable difference between groups of 20%, and (ii) the minimum number of animals required to provide descriptive results based on our previous experience. Most experiments require three replicates to demonstrate significant results. We

work closely with statisticians at UCL, who help us design experiments, which will conducted according to the ARRIVE guidelines, using randomisation and blinding where appropriate to avoid bias.

We aim to make optimal use of several tissues, fluids and cell types per individual mouse in order to maximise the information obtained from the minimum resource.

Refinement

We have chosen to work with mice as they are the lowest vertebrate group with well-characterised disease models.

Genetic modification of mice is well-established and their blood and immune system has been intensively studied and bears extensive similarities to that of humans.

The majority of reagents and tools required for the proposed plan of work have been designed for use in mouse models and for the detection and tracking of murine immune cells. To our knowledge no other species of lesser sentience can fulfil the requirements of this programme to the same extent as the laboratory mouse.

The proposed mouse models are already well established in our previous projects. All the protocols have well-defined humane endpoints and the majority of mice will be killed with an appropriate Schedule 1 method within 6 months of receiving gene-modified immune cells, or earlier, whenever endpoints are reached. All experimental procedures are carefully monitored by experienced staff.

Our experimental animals are monitored daily by our research group and we have excellent support from staff within the animal unit. Animals exhibiting any unexpected harmful clinical signs will be killed using a humane method, or in the case of individual animals of particular scientific interest, advice will be sought from the local Home Office Inspector.

Project 57	MYB PROTEINS AND MYELOID DISEASE SUSCEPTIBILITY
Key Words	Stem cells, bone marrow, blood cells, leukaemia, ageing
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;

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

Stem cells exist in most organs in our bodies where they serve as a continual source of new cells to replace any that are lost or die. All cells that constitute blood are produced throughout our lives from stem cells that reside in the marrow of our bones. These stem cells underlie bone marrow transplantations, which are commonly and effectively used to treat a range of blood disorders, especially malignant diseases such as leukaemia.

Blood formation from bone marrow stem cells is tightly controlled to ensure that the body has sufficient of the specialized cells to be able to respond rapidly and precisely to the need to transport oxygen, prevent bleeding and defend against infection. Failure to maintain this control can lead to cell deficiencies, particularly as we get older, or may cause the over production of cells and to diseases such as leukaemia. Key to achieving this control are proteins known as transcription factors whose role is to turn on and off those genes that are crucial to the production of the individual blood cell types. One group of transcription factors, the MYB proteins, are particularly relevant to the development of diseases of white cells and appear to play an important role in their age-related occurrence.

In this programme of study we will investigate how the fine balance of MYB proteins ensure that the stem cells in the bone marrow are maintained in sufficient numbers and that they correctly produce blood cells when necessary. We will also explore how, when this balance is not correct, lower levels of MYB proteins can lead to diseases in the blood cell system, including deficiencies or malignant conditions such as leukaemia. The way in which MYB proteins are involved in blood cell diseases that increase in frequency with age will be a particular focus.

We will use genetically altered mouse models to investigate how disturbances in the normal behaviour of MYB proteins can lead to these various diseases. These models have alterations in the MYB genes that lower or prevent production of the active proteins, either throughout an animal's life or at a specific time

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)?

This project will demonstrate how MYB proteins dictate the normal control of bone marrow stem cells and how they can be involved in the occurrence of both malignant disease and defects in blood cell production as we age. This new knowledge will help inform clinical practice and the development of strategies that manipulate MYB protein function for therapeutic benefit.

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

All studies will involve normal or genetically altered mice. The majority of the animals will be used in breeding to obtain mice with the correct genetic make-up for the provision of bone marrow tissue (approximately 20000). Other animals (approximately 7000) will be used in procedures to investigate stem cell function, especially by mimicking bone marrow transplantation, and the effects of ageing, which for a mouse means over the course of up to 2 years.

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?

The absence or loss of MYB proteins is expected to lead to a variety of blood disorders including deficiency in white cells and the occurrence of conditions that resemble malignant diseases such as leukaemia. Animals will be monitored regularly by blood sampling so that potential harmful consequences can be predicted, and animals can be humanely culled before they impact on their quality of life. Several of the procedures involve injection of substances, either to bring about deletion of MYB genes, as a means to track what is happening to the stem cells, or for the purpose of anaesthetising animals. These procedures may affect the animal either because of the nature of the substance injected or because of the injection process itself. Regular observation will ensure that any toxic effects of the substance injected are noticed quickly, and any animal showing any signs of pain, suffering or distress as

will be humanely killed. Injection can cause stress due to restraint and transient discomfort from needle insertion, but this will be minimised by good practice, animals being accustomed to the procedure whenever possible and handled quietly. Pain relief may be used in some circumstances. Any animals showing any signs of pain, suffering or distress as a result of toxicity will be humanely culled. Sampling of blood or bone marrow will be used frequently in order to monitor animals. This can involve some stress due to restraint and transient discomfort from needle insertion and blood collection, which can be mitigated by accustoming the animals to the procedure whenever possible and quiet handling. Direct sampling from the bone marrow requires that the animal is anaesthetised, and will also use analgesia to relieve the transient pain caused by the sampling needle. Exposure to ionising radiation is required in a number of procedures. This is either used to test how stem cells respond to damaging radiation through repair of their genome or, in the majority of cases that will be used in this programme, as a means to “condition” animals for the transplantation of stem cells. This conditioning destroys some of the resident stem cells in the animal receiving the transplantation, allowing the stem cells being tested to engraft. This technique is the gold standard for the testing of the capability of stem cells. Exposure to ionising radiation kills cells, the most susceptible organs being the bone marrow and the gut. Irradiation in smaller doses that add up to a larger dose significantly reduces any adverse effects. Transplanted animals also receive additional normal bone marrow cells to reduce the consequences of the loss of their own bone marrow. After irradiation, particularly around 1-2 weeks, animals may show symptoms of radiation sickness such as diarrhoea and loss of appetite, and will be carefully observed. If the symptoms persist or exceed an acceptable level then the animals will be culled by a humane method. Following irradiation animals are also at an increased risk of infection. This will be minimised by use of aseptic handling techniques, isolation, and the addition of antibiotics via the drinking water.

Application of the 3Rs

Replacement

It is only possible to gain a true picture of stem cell properties and the way in which disorders of the blood system develop, especially over long periods of time, and their overall impact can only be assessed in the context of a live animal. The modification of gene function and the testing of genetically altered cell function in vivo are not feasible in humans and can only be achieved in mouse models.

Reduction

Controlling the breeding colonies can have the greatest impact on reduction, ensuring that only the required numbers of animals are generated. This is achieved by careful planning and monitoring and by timely determination of the genetic make up of litters. Whenever possible, repeated sampling of animal models will be used to limit the numbers used. The numbers of animals required for observation of stem

cell behaviour can also be reduced if the cells are labelled so that they can be observed directly in live animals. This approach necessitates less frequent sampling of blood or the need to cull animals at specific time points for the analysis of bone marrow

Refinement

Experiments will be carried out using mouse as the species of choice for two main reasons. First, only in mice is it possible to perform genetic alterations to a specific gene. The use of such animals is critical to this programme of work as this is the only way to establish the role of MYB proteins in the normal functioning of stem cells in vivo. Second, the mouse offers the only model system in which it is possible easily to test stem cell function through transplantation. Our long experience in the use of these models has enabled us to refine the procedures in order to minimize the numbers of animals required through the breeding programmes, and to maximize the efficiency of use of those animals that can suffer moderate adverse effects so that they are utilized well in advance of any decline in their health. We maintain a database of all effects of the genetic alterations and associated adverse effects to help guide control measures and humane endpoints. Also, the procedures involving irradiation have been carefully optimized in terms of optimal degree of treatment, pre- and post-treatment care to alleviate infection and discomfort, and the definition of humane endpoints.

Project 58	Interrogating the Immunology of Cancer and the Development of Cancer Therapeutics
Key Words	Vaccine, therapeutics, cancer, immune cells
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;

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

The objectives of this project are:

1. Development of cancer vaccines to treat prostate, breast, ovarian cancers and glioblastoma multiforme (the most common and most aggressive cancer of the brain (GBM)).
2. Development of cell- based therapies for the treatment of prostate, breast, ovarian cancers and GBM
3. Determination of whether a protease is an efficacious agent for killing targets in cancer
4. Determination of whether the incorporation of pre-biotics and / or antibiotics and / or checkpoint inhibitors (which are drugs that cause an immune system to attack cancer cells) improve the efficacy of cancer vaccines and / or therapies.

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)?

Despite significant progress, cancer continues to be the leading cause of death worldwide. Prostate cancer is the most common cancer in men (1 in 8 men get prostate cancer in their lifetime). Breast cancer is the most commonly diagnosed cancer among women worldwide (522,000 women died from breast cancer in 2012). Glioblastoma multiforme (GBM) is the most aggressive form of primary brain tumours with an incidence of ~5/100,000 population. Ovarian Cancer around 7,300 women are diagnosed with ovarian cancer in the UK each year. The development of

new approaches for targeting cancer using the immune system, and a better understanding of the approaches which tumours use to protect themselves from immune attack will lead to the development of better therapeutic vaccines which will be taken to the clinic to benefit patients with cancer. The success of the therapies will be further improved through personalised approaches and in turn this will improve the quality of life for patients suffering from prostate, breast, ovarian cancers and glioblastomas.

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

Wild type mice, mice genetically modified to express elements of the human immune system and immunodeficient mice will be used to identify molecular features of cancers that can be used as the target for new vaccines. The capacity of these vaccines and other approaches to control tumour growth will also be studied. Immunoregulatory events and the inhibition of these will also be studied in these animals. Immunodeficient mice will be used for studying the capacity of new approaches to control the growth of human tumours. All experiments will be designed and performed in accordance with the 3Rs principles, in that animal usage will be minimised and experiments refined. We expect that the project will require approximately 16,000 mice over a 5-year period.

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?

All protocols have been designed in order to achieve the desired objectives without compromising the animal's welfare. The programme of research only proposes to induce tumours under the skin and these will not ulcerate or spread to other areas of the body or be allowed to prevent the normal behaviour of the animal or its movement repertoire. Although adverse effects such as transient discomfort from the immunisations and the presence of tumours may be expected, these are likely to be of mild severity. Adequate levels of anaesthesia and recovery from procedures will be ensured by close monitoring. The overall severity of the project is expected to be "Moderate", and all animals will be culled at the end of each study.

Application of the 3Rs

Replacement

Although this research employs an extensive portfolio of *in vitro* laboratory techniques, these cannot adequately model the complete array of biological and immunological events that are involved in tumour growth or the generation of protective anti-tumour immunity, all of which are important to our understanding of tumour immunology and the development of new, immune-based therapeutic strategies. *In vivo* studies in rodent models are therefore required.

Reduction

Studies will be designed to use the minimum number of animals that required for statistical significance and they will employ as much *in silico* and *in vitro* analyses as possible.

Mice that are genetically modified for multiple human HLA alleles (which are cell surface proteins that are responsible for the regulation of the immune system in humans) will be used whenever possible, rather than animals with single modifications.

Tumour cell lines will be maintained *in vitro* and stored frozen in order to negate the need for serial *in vivo* passage, thereby further reducing animal use

The proposed studies will monitor tumour growth using *in vivo* imaging systems which further reduce animal usage.

Refinement

The genetically modified (transgenic) mice expressing relevant elements of the human immune system that are to be used in the study will, as closely as possible, mimic the human situation with regards to the development of immune-based approaches for the treatment of cancer. The immune deficient mouse models are our strain of choice to test established vaccines and/or therapeutics.

The use of the *in vivo* imager will enable the delivery of an improved mechanistic insight into the responses that are being induced thereby allowing us to establish endpoints earlier. The therapeutic approaches that are to be developed are minimally invasive and are expected to exhibit minimal adverse effects

The use of *in vivo* imaging systems provides robust scientific data which can be acquired sequentially in the same animal.

Project 59	Pancreatic cancer – improving our understanding and therapeutic options
Key Words	Pancreatic cancer, Therapeutic, Genotype/Phenotype
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;
Yes	(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);

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

The aim of this project is to improve the dire prognosis of individuals with pancreatic cancer. We will do this using patient tumour samples which have been sequenced to gain an understanding of the genetic events driving the cancer, and determine the tumour genetics which predict response to therapy. We will also utilise genetically engineered animals to investigate the role of particular mutations frequently found in cancer, in tumour initiation, progression, and metastasis, and to determine their response to therapies. Using genetic information, we will also determine which of the multitude of mutations acquired by tumours are actually responsible for growth and metastasis, in order that therapies can be focused on the appropriate genetic lesions.

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)?

This project will result in gaining a better idea of which patients (with specific genetic mutations) should respond to particular therapies, thus enabling treatment to be targeted to those who will benefit from it. Furthermore, by performing simultaneous in vivo and in vitro experiments from the same primary tumour, we hope to draw

conclusions as to which model system best recapitulates the human situation with respect to response to therapeutics. Additionally, by beginning to examine the effect of different mutations, we can identify driver vs passenger mutations, thus determining which pathways/molecules are best targeted for therapy.

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

Over a period of 5 years, we expect to use up to 30000 mice.

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?

The majority of animals will experience few or no adverse effects. Most animals will not undergo any surgical procedures beyond sub-cutaneous implantation. The therapeutics being used are predominantly already in clinical use or clinical trials, meaning that the effects are known and recognised, and can be mitigated through support during treatment. All animals are to be humanely killed at the end of each study, or if they do not respond to therapeutic or supportive care.

Application of the 3Rs

Replacement

Cancer is a disease of mammals, and cannot be fully recapitulated using in vitro models. Using mice enables us to examine the effects of vasculature, multiple cell types, the immune system, cytokine signalling, etc, on the tumour cells, and their growth. We still do not fully understand cancer as a disease, nor its systemic effect on the body, and we cannot model what we do not know

Reduction

Statistical analyses have been performed to determine the minimum number of mice required to give statistically valid results, thus minimising the animals used, and the repeats required. Breeding will be set up to optimally generate the genotypes of interest, thus decreasing the number of animals which are not of interest genetically. Experiments will be combined where possible, to minimise the numbers of animals required as controls between studies

Refinement

Mice are the only species used in this programme of work. They are well characterised with respect to their similarities (and differences) to human disease. Using genetically altered models enables us to closely recapitulate human disease, and patient-derived xenografts enable us to reproduce the original human tumour with little distress to the animals. Mice will be regularly monitored, and humanely killed if they develop signs of distress.

Project 60	Vaccine Development and Immunotherapy
Key Words	Cancer Therapy, Vaccine, Therapeutic antibody
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;

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

Objectives of this project are:

1. To identify targets within melanoma, breast, lung, ovarian, pancreatic and colorectal cancers for immune therapy.

2. To develop cancer vaccines to treat melanoma, breast, lung, ovarian, pancreatic and colorectal cancers

3. To develop antibody therapies to treat colorectal, pancreatic and gastric cancers

4. To determine if combination therapies improve cancer therapy.

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)?

Cancer directly affects 1 in 2 of the population during their lifetimes and although significant progress has been made in the last 10 years cancer still remains a major cause of mortality and improved therapies are required. Melanoma is the 5th most common cancer in the UK with approximately 2,500 deaths each year. Lung Cancer is the 3rd most common cancer, with an estimated 35,800 deaths every year. Pancreatic cancer is the 10th most common cancer in the UK, with 9,400 new cases diagnosed and 8,800 deaths each year. This cancer is almost always fatal. Colorectal cancer is the fourth leading cause of cancer-related mortality worldwide and accounts for over 15,000 deaths annually in the UK. Ovarian cancer is the 5th most common cancer in women accounting with around 7,300 people diagnosed and

4,100 deaths in the UK each year. Breast cancer is the most common cancer in women with 53,700 new cases and 11,400 deaths each year in the UK. The scientific insight and understanding generated by this project will help broaden the understanding of how the immune system can interact with and target cancer and how best to focus the immune response against cancer. This will result in the development of vaccine strategies and other immunotherapies that effectively target these cancers and reduce the number of cancer-related deaths

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

We will use wildtype and genetically altered mice and rats expressing components of the human immune system to study the immune responses to cancer, immune pathways involved in cancer therapy and the capacity of vaccine and therapeutic antibody treatments to control tumour growth. We expect to use approximately 9000 mice and 50 rats in this project over a 5 year period. All experiments will be designed and performed under the 3Rs principles in that experiments will be Refined and animal use Minimised.

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?

All protocols have been designed to obtain the desired objective without compromising the animal's welfare. Protocols are designed to assess the immune responses to vaccine candidates and generate therapeutic antibodies. Although animals are likely to experience transient discomfort from vaccinations and presence of tumour implants, these are expected to be of mild severity. Tumours result in small round nodules of no more than 15mm in size and are not expected to compromise mobility or normal behaviour of the animals. Anaesthesia will be used in certain procedures. Appropriate anaesthetic levels and recovery will be closely monitored. The overall level of severity is expected to be moderate, and animals will be culled at the end of each study.

Application of the 3Rs

Replacement

This research also makes use of a variety of *in vitro* laboratory techniques whenever possible. However, *in vitro* assays cannot adequately model the complete array of events involved in tumour growth, or the generation of an immune response, both of which are essential to our understanding of tumour immunology. *In vivo* studies using rodent models are therefore crucial.

Reduction

Studies will be designed to use the minimum number of animals required for statistical significance.

Candidate vaccines will be designed with use of *in silico* and *in vitro* techniques prior to evaluation in animals.

Mice with multiple rather than single relevant genetic modifications will be used whenever possible.

Tumour cell lines will be maintained *in vitro* and stored frozen to negate the need to use additional animals for maintenance *in vivo*.

In vivo imaging platforms will be established and used to sequentially monitor tumour growth in animals enabling the animal to be its own control

Refinement

Genetically modified mice used in this study are the closest representation for the human immune system for vaccination studies and tumour models have been engineered to use in these strains.

Vaccination strategies that are minimally invasive and optimised to minimise adverse effects will be used in preference.

In the assessment of tumour therapy subcutaneous tumour models will primarily be used as these are the least invasive and provide the least harm to the animal.

This involves injection of a small number of cells under the skin on the flank which form a distinct small round nodule up to 15mm in diameter. The placement on the flank is least invasive and least likely to compromise movement or normal behaviour.

The use of tumour cell lines in conjunction with *in vivo* imaging platforms will enable more efficient and accurate sequential monitoring of tumour growth and anti-tumour immune responses in the same animal.

Genetically altered mouse and rat strains rather than wild type animals will be used for the generation of fully human therapeutic antibodies, as these are of greater clinical potential and will retain better anti-cancer efficacy.

Project 61	Regulation of tumour growth and metastasis by sodium channels
Key Words	Antiepileptic drugs, Breast cancer, Invasion, Metastasis, Sodium channels
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;

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

The goal of this research is to gain a greater understanding of a type of ion channel that exists on the surface of breast cancer cells, called a VGSC, or 'voltage-gated sodium channel'. This sodium channel opens when there is a change in the cell's membrane voltage and allows sodium ions to flow into the cell. We have found that VGSCs are present on breast cancer cell lines cultured in the laboratory, where they help the cells to move and invade. These proteins are therefore potential new targets for the treatment of invasive breast cancer. The plan is to study the role of VGSCs in regulating breast cancer metastasis in mice. We use therapeutic drugs to inhibit VGSC activity, and genetic approaches to switch VGSC genes on or off in breast cancer cells.

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)?

Breast cancer is a leading cause of death worldwide. The major cause of mortality in breast cancer is metastasis, the process by which cancer cells spread from primary tumours to secondary sites. There is an urgent need for more effective treatments to combat metastasis. VGSCs allow sodium ions to pass into/out of neurones. Drugs that target these channels are used in patients in order to treat epilepsy, abnormal heartbeat and pain. VGSCs have been detected in a range of human cancers. The main potential benefit of this work is that VGSCs may be alternative targets in breast cancer diagnosis and therapy. VGSC-targeting drugs already in clinical use might

also be effective in breast cancer treatment. By better understanding the role of VGSCs in regulating metastasis, we should be able to design new, better treatments in order to reduce and/or slow breast cancer metastasis.

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

Mice, 1800 animals over 5 years.

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?

The surgical procedures experienced by the mice in this project will be of no more than moderate severity. A number of the mice used in this project will develop breast cancer. The tumours may grow to a size big enough to cause minor discomfort, in which case we will give the mice pain-relieving medication. Mice will be put to sleep at the end of the experiment and their tissues will be banked for analysis and to reduce the need to use further animals.

Application of the 3Rs

Replacement

Our laboratory uses *in vitro* models as investigative tools whenever possible, and a large part of this work uses *in vitro* tissue culture techniques. However, at this point it is necessary to use animals to answer questions about VGSC function during metastasis *in vivo*. Metastasis is a complex, integrative process that cannot be accurately mimicked using cell lines or computer models alone. Cancer studies in animals yield valuable insights into our understanding of metastatic breast cancer.

Reduction

We use *in vitro* models and clinical datasets wherever possible in order to limit the number of animals used for this research. We use state-of-the-art imaging approaches, which enables us to generate large amounts of data on tumour growth and metastases from individual mice, reducing the numbers needed. Finally, we collect and bank tissue from the animals used in this project and share these samples with the breast cancer research community in order to reduce the number of animals used in breast cancer research.

Refinement

Mice are unique in their ability to accept, develop and accurately model breast cancers. These models involve the injection of human cancer cells into the breast of female mice, or using genetically modified mice that spontaneously develop breast cancer. The tumours may grow to a size big enough to cause minor discomfort, in which case we will give the mice pain-relieving medication. The tumour, metastases and the response to treatment, can then be seen using specialised imaging systems

and microscopes. Some of the mice will be given a non-toxic dose of a VGSC-inhibiting drug with or without other chemotherapy. Some of the mice used in this project are immunodeficient so that they can accept human tumours. They will be kept in individually ventilated cages to avoid infections.

Project 62	Immune cell mechanisms in cancer and infection
Key Words	Infection, Cancer, Immunology
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;

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

Cells of the immune system are critical to defend the body against cancer as well as infection by viruses, bacteria, and parasites. In most circumstances, these immune cell types are carefully regulated ensuring that appropriate responses are made during infection and that following clearance of pathogens, the immune response returns to a resting state. However, in some cases, inappropriate immune activation can result in autoimmune diseases such as Type 1 diabetes or rheumatoid arthritis. On the other hand, immune responses are often defective in cancer allowing tumour growth and spread. Therefore, the overall objective of the project is to investigate the molecular mechanisms that regulate the immune response in these different settings.

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 studies are designed to identify molecular targets that are essential for normal immunity and to determine the impact of interfering with these pathways on immune responses to cancer and infection. By identifying pathways and mechanisms involved in immune cell activation, this information may provide insight into new therapeutic approaches, e.g. novel therapies to improve immune responses to cancer. Therefore, we have two main aims; 1) to determine the role of specific molecules in immune responses 2) to identify potential therapeutic targets that could be used to enhance (in the case of cancer) or decrease (in the case of autoimmunity) immune responses.

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

Our studies exclusively use mice and we anticipate using approximately 7250 mice over the 5-year time-frame of this project.

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?

All procedures to be carried out are associated with a 'mild' or 'moderate' severity rating. Under the terms of this license, animals will be euthanased at the end of all experiments.

Application of the 3Rs

Replacement

Immune responses to infection and cancer are tightly regulated and involve numerous cell types and organs. As non-animal alternatives can not replicate the full complexity of immune responses in intact animals, in order to best gain insight into how we might manipulate these responses in future therapy, we have chosen to use the laboratory mouse as our model system.

Reduction

Our previous extensive experience of animal research has allowed us to develop robust protocols involving the minimum numbers of animals required to provide reliable and informative results. Importantly, we routinely seek advice from statistician colleagues in order that our experimental design can be optimised.

Refinement

The availability of resources for genetic manipulation underlies the choice of the laboratory mouse for this programme of work. Furthermore, a wealth of commercial reagents and techniques for analysing and manipulating immune responses in mice are available. Over many 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.

Mice will be monitored regularly and routinely for signs of ill health or distress throughout all aspects of the project. Where appropriate, anaesthetics and pain-killing drugs will be used whilst advice from local veterinary surgeons will be sought in any situation where animals are showing unexpected signs of ill health or suffering. In all cases, experimental protocols have pre-determined “endpoints” that when reached, animals will be removed from the study.

Use of non-invasive *in vivo* imaging can also pick up progression of pathological changes in longitudinal study in one animal. This technique allows us to use humane

end point in consistent manner thus increasing experimental reliability and reduction of animal use.

Project 63	Immune and Biological Therapies for Cancer
Key Words	cancer, virus, immune system
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;

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

We aim to develop novel treatments for cancer using viruses that activate the immune system and can also kill cancer cells directly. We will also look at the effect of viruses on other cells within the tumour as these cells often contribute to cancer progression and therefore may be a useful target in treating the disease.

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)?

We aim to develop new treatments for cancer that can be transferred into the clinic and lead to improved outcomes for patients. Viruses that target cancer cells and the immune system have already been trialed in patients and we aim to develop new viruses that can improve outcomes.

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

We anticipate using 4000-5000 mice over the 5 years of the project. Most of these will be wild type animals but a small number will be immune-deficient as this will allow us to identify the mechanisms involved in successful treatments and hence lead to further improvements

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?

The animals will be inoculated with tumour cells which will be allowed to grow and form established tumours. These injections and the resulting cancer masses may cause discomfort. Tumour bearing animals will receive treatment with virus, drugs or radiation therapy. Viruses and drugs are administered by intratumoural, intraperitoneal or intravenous injection or by gavage and radiotherapy is delivered under anaesthetic. Again, these procedures may cause some discomfort. Tumours are measured in order to assess the effects of therapy. The animals are killed humanely at the end of the experiment and are expected to suffer no more than a moderate degree of adverse effects during the entire experiment.

Application of the 3Rs

Replacement

Although we conduct many experiments *in vitro* to investigate the immune responses induced by our viruses these are not fully representative of what may happen in patients. Immune responses involve complex interactions between many immune cell types and these take place at different locations in the body. Therefore *in vitro* experiments can only give a limited amount of information regarding potentially useful treatments. For this reason we need to test our therapies in animals that have a fully functioning immune system

Reduction

The number of animals used will be minimised by conducting *in vitro* analyses before proceeding to test our data in *in vivo* studies. We will also estimate the size of the likely therapeutic effect in order to perform statistical power calculations that will avoid designing experiments with group sizes that are too large.

Refinement

The rodent tumour models used are the lowest form of mammal recognised as relevant for human cancer.

Mice do not develop spontaneous cancer at a rate compatible with experimentation, therefore tumour cells will be implanted into the mice. Most studies will be performed on animals with subcutaneous tumours. However, other experiments will model tumours growing in more appropriate sites e.g. intraperitoneal for ovarian cancer and intracranial for brain tumours. Our work is based on humane treatment of animals at all times. We have a full-time qualified Animal Technician to ensure the highest standards are maintained in our work. Discomfort and distress experienced by the animals will be limited to unavoidable procedures required for the conduct of sound research. If at any time an animal is found to be showing signs of ill health it will be killed humanely.

Project 64	**MODELLING A GENE FAMILY IN HUMAN DISEASE**	
Key Words (max. 5 words)	cancer heart skin inflammation	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our laboratory has identified three related genes and the work carried out by ourselves and others has implicated their importance in development, cancer and human disease. To understand how they function in vivo, we have generated mice carrying mutations in these genes, and this has revealed that our transgenic mice may serve as experimental model systems for human disease. One of our genes is involved in regulating the junctions between cells in the epithelia lining organs like the gastrointestinal tract and the kidney. 90% of human cancers are epithelial in origin and we have evidence that this gene is a tumour suppressor, and under this licence we will further test this by giving our mutant mice cancer-causing stimuli to assess whether there is a difference in the cancer rates when compared with wild type animals. There is also a role for this gene in brain development, and mice lacking	

	this gene may be a model for neuronal tumours. This gene may also participate in the response to low oxygen, which will also be investigated. Mice lacking another of our genes have defects in the heart and skin, analogous to a genetic condition seen in humans and cattle which can result in sudden death. In this licence the links with these inherited conditions will be further characterised. The exact nature of the heart defects in these mice will be characterised, and the role of this gene in wound healing will be examined by measuring the healing rate of a dorsal skin wound. Also, a common side effect of cancer therapy is cardiotoxicity, and whether our mice with this heart defect can also be used to analyse cardiotoxicity in response to chemotherapeutic drugs will be investigated. We have less information about our third gene, but existing studies have shown that all three members of this family, but this member in particular, function in the immune system and may impact upon the response to physical injury or pathogens. The immune systems of the mice will be tested by performing bone marrow transplants to analyse the growth of stem cells and skin allografts to determine the ability of the mice to reject transplants. Around 20% of cancers are associated with chronic inflammation, often resulting from lifestyle factors such as diet, alcohol consumption and smoking. Specific examples include: the links between gastric reflux and oesophageal cancer; chronic pancreatitis and pancreatic cancer, and the presence of the bacteria *Helicobacter pylori* in the stomach lining and gastric cancer. Work performed under this licence aims to develop and use mouse models to mimic these diseases with the intention of both gaining a better insight into these cancers and developing new therapies to alleviate these conditions.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	The ultimate aim of this plan of work is to establish mouse models of human diseases and use these to identify effective treatment strategies to benefit patients.

project)?	During the period of our previous licence, we unexpectedly identified a novel mouse model for a form of human congenital heart defect. In addition to contributing to our wider knowledge of how the heart develops and functions, we are optimistic that, in association with clinicians, mutations akin to those carried by these mice will be identified in patients. This could lead to the development of genetic tests that can be used in the clinic to identify carriers of this condition. Under this licence, we aim to develop models for human cancers in organs such as the pancreas, oesophagus, stomach and colon. There is an unmet clinical need for treatments for these cancers, especially as the 10 year survival rates for oesophageal and pancreatic cancers are among the lowest. It is hoped that these models and the data that they yield will be of interest and use to other researchers in the field. We also hope to use these models to test novel therapies for these cancers. In addition to generating disease models, our transgenic mice have the potential to further our understanding of embryonic development, epithelial biology, tumour development and cardiac function. Therefore, this study will shed light on the signalling pathways in which our genes participate, and the knowledge generated should be of interest to the basic science communities working on cell signalling. We intend to publish our findings, and we have a very strong publication track record which we are confident will continue in the future.
What species and approximate numbers of animals do you expect to use over what period of time?	All our work uses mice. We plan to use a maximum of 36850 animals over 5 years for this plan of work. The majority of these animals will be used for breeding.

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 order to mimic human pathologies, the procedures the mice undergo will inevitably reflect the human experience. For all our mice, we plan to use well-characterised, standard experimental techniques that should not cause more than a moderate degree of suffering. However, the majority of our mice will be used for breeding or post-mortem analysis and will undergo no more than mild discomfort. In many instances the genetic alteration of our animals will cause no adverse effects. With respect to our mice carrying congenital abnormalities, e.g. heart defects, we are able to identify these mice at an early stage and carry out our analyses while they are still in good health. All surgical procedures will be performed aseptically under general anaesthesia and pain relief will be given. To analyse the rate of wound healing some mice will have one or two 4-6mm wounds created in the skin on their backs, which will then be allowed to heal. In our experience the wounds heal within a few days and the wounds do not distress the animals. Similarly, we will graft skin onto mice to test immune response, and again pain relief and asepsis will be employed. Where the immune system is compromised the animals will be kept in clean conditions to minimise the chances of infection. Some monitoring procedures, for example analysis of tumour growth or cardiac function, will be carried out under general anaesthetic. Some animals will be exposed to reduced oxygen levels. The mice will either experience short periods of low oxygen or a gradual reduction over 1-2 days. This rarely causes the animals any distress although they may undergo transient weight loss. Many of the methods used to initiate tumours in this licence are non-invasive, e.g. the exposure to X-rays, by the application of non-irritating chemicals to the skin, or the inclusion of chemicals in the drinking water or diet. Other methods require one or two injections. In instances where multiple injections are required, we will endeavour to find alternative methods that improve the experience for the animal.

	Once the animals have been given a carcinogenic stimulus they will be closely monitored for signs of tumour growth and will be humanely killed as soon as any signs of suffering, such as breathing difficulties, are identified. Ultimately all the mice will be killed in a humane manner, and frequently their tissues will be used post-mortem for further analysis by biochemical or histopathological methods.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Many of our initial observations have been made using in vitro culture systems and they frequently inform our in vivo experiments. We continue to use cell culture techniques involving tissue taken from our animals post mortem, so that we can manipulate the cells without having to use a live animal. However, there are currently no means of accurately recapitulating in cell culture the many complex interactions that occur between multiple cell and tissue types in vivo, many of which are still uncharacterised. Therefore, to most accurately mimic human disease we need to use animals in our research.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Where possible we cryopreserve any strains of mice that are not being actively used. We also use Cre-Lox technology which frequently reduces the number of spare mice that are generated by standard Mendelian crosses. Where necessary, before undertaking an experiment we will perform a statistical analysis to ensure that the correct numbers of animals are used to enable robust conclusions to be drawn.
3. 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	Mice are the lowest species of mammal which can be used to study development and cancer. Mice are the only mammal in which transgenic technology works reliably, and their genome, biology and physiology has been widely studied and documented. Mice are routinely housed in IVC cages to minimise the risk of infection and supplied with environmental enrichment. We regularly use Cre-Lox technology which

(harms) to the animals.	conditionally alters gene expression, so instead of occurring throughout development, gene alteration can be made to occur only in specific tissues, upon maturity or post-mortem in cell culture. Where mice have an adverse phenotype when homozygous, the strain will be maintained as heterozygotes where possible. Where surgical procedures are performed analgesia will be given to minimise discomfort and strict asepsis will be observed. When novel procedures are undertaken, pilot studies will be performed initially, and the outcomes of these will be used to amend future work. When using known carcinogenic stimuli we will use doses that cause the minimum impact upon the controls while still giving an effect upon the experimental subjects. Tumours will be allowed to grow only until the minimum amount of tissue required for analysis can be recovered. Some of our studies may predispose animals to develop diabetes, so we will check this by testing urine, which is non-invasive. We also plan to trial more refined methods, e.g. the replacement of multiple injections with continuous dosing from a mini pump, or the reduction in the diameter of the skin wounds created to analyse healing.

Project 65	Cancer Therapy – Enabling Licence
Key Words	Tolerability, Pharmacokinetics, Tumour, Surrogate
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;
Yes	(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);

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

The overall aims of this project licence are (1) to provide data necessary to identify suitable *in vivo* candidates for progression into disease and/or surrogate models of cancer and (2) to identify and develop suitable models of disease or physiology which will be used subsequently to evaluate new and improved cancer treatments.

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)?

The benefit of this project will ultimately be the introduction of new and improved therapies for the management of cancer. This project licence will enable therapies to be identified as suitable in vivo candidates for progression into disease and/or surrogate models of cancer. This licence will also identify and develop suitable models of disease which will be used subsequently to evaluate new and improved cancer therapies.

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

Only rats and mice will be used on this project. Total number of mice used over 5 years will be 24,500 and the total number of rats used over the 5 years will be 9000.

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?

Clinical signs related to the pharmacological action of the compound may be seen and mild to moderate signs of toxicity are possible. Animals will be humanely killed if this persists. Local irritation at the site of injection may be observed. Animals will be closely observed on the day of dosing. Animals are observed by trained staff, with referral to the Named Animal Care and Welfare Officer, veterinary staff and Project Licence Holder as necessary. All animals will be regularly monitored for weight loss and general condition. Weight loss as a result of repeat anaesthesia may occur and this will be minimised by correct dosing, accurate weighing and good maintenance of body temperature during the period of anaesthesia and the recovery phase. Animals that are used where the immune-system is compromised will be housed in sterile conditions. For tumour studies adverse effects related to tumour inoculation may cause brief discomfort or pain. Adverse effects will be minimised by: • limiting volumes • choice of appropriate needle size • application of good technique by trained licensees The tumour types used are very well tolerated and only one superficial tumour will be used per animal. Tumour size and condition is monitored closely on a daily basis and we will use the least invasive tumour site/line that will achieve the scientific aims and will apply the earliest endpoints to meet the scientific requirement of the study. Animals will be culled if the tumour results in significant pain or distress. The protocols are classified as moderate severity. Animals will be humanely culled at the end of the study. Weight loss as a result of repeat anaesthesia may occur and this will be minimised by correct dosing, accurate weighing and good maintenance of body temperature during the period of anaesthesia and the recovery phase. Animals that are used where the immune-system is compromised will be housed in sterile conditions. The protocols are classified as moderate severity. Animals will be humanely culled at the end of the study.

Application of the 3Rs

Replacement

Non-animal alternatives are used in the identification and selection of compounds and generally include measurements of the likely effect of the agent on the target cells. Activity in particular cell types however, cannot predict the likely *in vivo* activity given the complexity of issues such as bioavailability, metabolism and elaborate physiological interactions associated with tumourgenesis and therefore the whole animal is needed for the studies proposed in this licence.

Reduction

To maximise the scientific integrity of data generated and to use the minimum number of animals, in house statistical expertise will be applied to all experimental

design and analyses. Where plausible the following statistical guidelines will be used to minimise the number of animals required for each procedure:

- meaningful biological change and measurable endpoints will be defined
- estimates of biological variability will be used in sample size and power calculations
- animals will be allocated in an optimal way based on estimates of biological variability established from accrued historical databases, pilot studies or published data.
- regular monitoring and updating of biological databases with regular review of group sizes.
- one-sided (rather than two-sided) statistical tests will be used wherever appropriate (e.g. when identifying inhibition rather than change)
- statement of intended statistical analyses and justification for use, if any, of transformed data (e.g. tumour growth data may be analysed on the logarithmic scale if the variance of tumour measurements increases with the mean)
- statistical power will be set to a minimum of 80% (e.g. at least an 80% chance of declaring the defined 'meaningful biological change' as being statistically significant)
- multiple treated groups will be compared against one control to reduce the number of studies performed. Group sizes may be weighted to reflect this.

For each and every experiment, as part of Good Laboratory Standards, an experimental protocol is written which includes:

- a statement of the objective(s)
- a description of the experiment, detailing experimental treatments, the size of the experiment (number of groups, number of animals per group), duration of experiment, scientific endpoint

Every experiment conducted will have a Statistical Health Check associated with it which has been signed off by a statistician.

Refinement

The overall plan of the work is to support the discovery and development of novel cancer therapies to benefit human health in the treatment of cancer. Only rats and mice including immune-deficient strains are used on this licence. Using non-mammalian species of lower neurophysiological sensitivity is not possible since they lack appropriate tissue physiology. Although exact replication of all pharmacokinetic parameters between species is not possible, many features of human PK can be predicted from those observed in small mammalian species unlike effects seen in lower organisms.

The most appropriate species and strain of mice and/or rats will be chosen based on previous data that has been used to generate single agent efficacy data. Mice will be

used in the majority of studies unless there is a scientifically relevant reason that mice cannot be used, for example, compound metabolism issue with the compound. The choice of strain will be driven by the choice of tumour model. For human tumour lines immune-deficient animals are required to support the growth of the tumour, the least immune-deficient strain required to promote good, reproducible tumour growth will be used. The optimal conditions for tumour growth will already have been developed.

For anaesthetic protocols the optimal anaesthetic regime relevant for the species/strain will be developed and used in conjunction with the NVS.

Where necessary, pain relief will be used under the guidance of the NVS.

Project 66	Sarcoma, bone niche, microenvironment and therapy
Key Words	Cancer, Microenvironment, Biomarkers, Therapy
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;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;

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

This project aims to determine how a tumour cell finds a favourable environment (niche) in the body to grow and multiply and form a cancerous tumour. Our current knowledge reveals that bone is a favourable environment for tumour cells to target and take up residence. However, we do not know why that should be the case and therefore this project aims to find out what signals allow this to happen. If we could find these signals we could either block them with a novel drug or therapy, or alter this environment such that it does not promote tumour growth and therefore prevent the cancer developing.

Sarcomas are rare cancers that behave as described and have a 5-year survival rate of less than 20% for non-localised tumours to around to 70-75% for localised tumours. This 5-year survival rate for sarcomas has not significantly changed for the 4 last decades. Therefore, our drive in this project is to identify targets such that therapies can be developed to increase these survival rates.

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)?

The projects proposed will improve the knowledge on the bone remodeling and the pathogenesis of bone and soft tissue sarcomas in order to : i) identify new biological markers of recurrent disease, ii) identify new therapeutic targets, and iii) propose new therapeutic approaches. A better knowledge of the tumour niche associated with bone sarcomas will also open new therapeutic ways for other bone remodeling disorders (osteoporosis, prosthesis loosening, etc).

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

- Species: mouse - Number of animal: 6250 - Period of time: 5 years

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?

The mouse models that will be used in this project have been established during the course of previous studies and the severity will never exceed moderate for any procedure/protocol. In our project: Approximately 60% of the animals will undergo procedures to induce a cancerous tumour. Animals will be given pain relief to minimise discomfort and close monitoring will minimise distress. We will also follow established guidelines to study tumour models that ensures animal welfare is paramount and tumour growth is the minimum necessary to achieve the scientific aims. Tumours will not be allowed to exceed limits on size or adverse health impacts. . Procedures continue to be refined where earlier time points may be used, therefore minimising any distress to the animal. As part of this project we will also use genetic models of tumour formation, where these animals have been shown to develop tumours spontaneously and therefore mimic more closely the human disease. All animals that either have tumours induced, or arise through their genetic make will be monitored closely and humanely killed at the end of the experiment. Animals will be housed in appropriate social groups in cages that are environmentally enriched in a manner appropriate for the species. Husbandry and care will be based on best practice and veterinary advice and will be performed by highly trained and competent staff. All animals under procedure will be frequently and closely monitored and if health problems are observed veterinary advice will be sought.

Application of the 3Rs

Replacement

Modelling the whole interaction between tumour cells and the environments in which they grow in patients is not possible in cell culture, hence the need to use mouse as the model organism to study tumour biology. However, in parallel to our animal studies we will use cells in culture from both animal and human to aid our studies. In addition we work closely with computer modellers to develop both our *in vivo* and *in*

vitro approaches to help develop computer simulations that may inform if a therapy or treatment is likely to work or not.

Reduction

The generic experimental design to be used in all three objectives described is based on experience, the variance of previous quantitative published data and in consultation with the Statistical Services Unit of our Institution.

The use of Genetically modified mice (e.g., mice expressing a fluorescence colour in their developing tumour) will reduce the number of animals required per study as one will be able to follow the tumour development using imaging equipment that is able see this tumour growth in the live animal over a period of time. Longitudinal studies will also contribute to the significant reduction of animals used.

Refinement

Various animal models have been described in the literature for studying bone remodelling and sarcomas (fish, rat, mouse, dog). Fish especially Zebra fish is more adapted for studying cartilage than bone development and can be used to analyse the interaction of cancer cells with endothelial cells and the extravasation process. Fish model can not be used to analyse bone cancer environment and lung metastases. Large dogs suffer frequently from bone sarcoma and soft-tissue sarcomas are also observed in different breeds. However, the diversity of dogs and their various physiological parameters make difficult their used as model of bone and cancer diseases. Dog models are considered as models of spontaneous diseases adapted for clinical trials. Rat and mouse are frequently used in scientific research. Both species are relevant to study bone diseases and sarcomas. However, the limited number of biological tools available in rat (e.g. cancer cell lines, specific antibodies) makes difficult their use in the project. Mouse models allow the investigation on human diseases by using xenografts, mimic the human pathologies (e.g. osteoporosis with a dysregulation between osteoblasts and osteoclasts, development of lung metastases and alteration of bone remodelling in bone sarcomas). Genetically-modified strains are relevant and necessary tools to analyse the role of specific proteins on bone remodelling and their impact on the cancer niches. In this context, mouse models are the most relevant and adapted approaches for investigating bone diseases and sarcomas.

The mouse models used have been established during the course of previous licences to study local and systemic bone loss and tumour development. We have already identified the time course of bone loss events, of primary tumour growth and initiation of lung metastases. We continue to refine these measurements such that we can intervene at earlier time points, therefore minimising distress to the animal. The use of genetically modified models developing spontaneous tumours will reduce the need to inject and handle each animal.

Regular monitoring, the use of analgesics and dedicated husbandry staff will be used to minimise suffering.

Project 67	**Hematopoiesis in Development, Aging and Malignancy**	
Key Words (max. 5 words)	Stem Cells, Development, Leukemia, Aging, Transplantation	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	As humans age, their likelihood of developing acute myeloid leukaemia (AML) increases from <20 cases/million people/year to >200 cases/million people/year, and the likelihood of being cured of the disease decreases from 30-40% to less than 20%. Conversely, acute lymphoblastic leukemia (ALL). ALL is more common in young children (>1000 cases/million/year), decreasing to <100 cases/million/year in the elderly population, and has a much better prognosis. We know little about how blood forming stem cells change as we age, or about which subsets of stem cells give rise to different types of leukaemia, including AML. In this project we will - study the normal formation and regulation of blood forming stem cells, how their numbers and behaviour change during aging - identify the stem cells and progenitors giving rise to malignant blood diseases, and the reasons the	

	frequency of disease development increases with age. - develop molecularly directed therapies against acute leukaemias, in particular AML
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)?	These studies will help to improve human health in several areas: 1. Understanding the formation, diversity and regulation of normal blood forming stem cells will help improve therapies (congenital disorders, cancer) where bone marrow transplants are necessary for cure. 2. Identifying the cellular and molecular basis for how the blood system ages will allow us to rationally generate therapeutic interventions to counteract the decline in lymphocyte and red blood cell production associated with human aging. 3. Develop molecular therapies to prevent the development of acute leukemias, and to eradicate AML, a disease with a highly adverse prognosis.
What species and approximate numbers of animals do you expect to use over what period of time?	25000 mice over 5 years
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 majority of mice will be used to generate genetic models (gene knockouts) for the study of how blood forming stem- and progenitor cells are specified and regulated. These studies are not expected to be associated with adverse effects, and mice will be humane killed at the end of the experiment. A small proportion (ca. 10%) of mice may experience adverse effects as a result of the genetic modification, including leukaemia development. This severity will not exceed the moderate limit, and monitoring is in place to ensure this limit is not breached. Mice will be killed if they are observed to be suffering, or deemed likely to start to do so, or at the end of the experiment. Mice used for the study of aging may occasionally (5-10% of animals) experience health deterioration. A

	specific protocol is in place for monitoring the development of adverse effects associated with normal aging, and mice will be killed if they are at risk of exceeding moderate severity, or at the end of the experiment. For studies of stem cells and leukemic stem cells transplantation studies will be used. These may be associated with adverse effects due to irradiation in a minority of animals (<5%). Mice transplanted with leukaemic cells may develop disease, and will be monitored as described above. Mice will be killed at the end of the experiment. In order to minimise the number of animals used, and maximise the relevance of the results obtained to human health, we will generate disease models that mimic human disease as accurately as possible, and use statistical calculations to determine the minimal number of animal required to reach a from conclusion in our experiments. A very minor fraction of the genetically modified mice (<1%) may be transferred to other projects with authority to use genetically modified mice.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Stem cells and progenitors cannot be stably maintained ex vivo, and we cannot mimic aging outside the organism at present. Likewise, leukaemic cells cannot be reproducibly maintained in culture.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use the most sensitive analytical techniques (e.g. microfluidics-based gene expression analysis; multicolour FACS) available to obtain the maximum information form the fewest possible animals. Breeding strategies will be optimised to minimize animal numbers required. Cryopreservation will be used to maintain strains not in use.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	The genetic models used are designed to be accurate in mimicking human disease, to identify and highlight specific stem- and progenitor cell subsets, and to introduce genetic modifications in a tissue-specific

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	manner whenever possible. The results obtained will therefore be maximally informative about the problem under study. State-of-the-art husbandry (IVC cages, highly trained staff and veterinarians) will be used to ensure animal welfare. Monitoring systems are in place to control adverse effect. Drugs and biologically active agents will be administrated by the least invasive route appropriate for the study (e.g. orally rather than by intraperitoneal route in the case of tamoxifen).

Project 68	***In vivo* evaluation of enadenotucirev derived viruses**	
Key Words (max. 5 words)	Oncolytic, Adenovirus, Cancer, Immunotherapy	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	✓	Translational and applied research
	✓	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The key objective of the programme of work is to progress 'armed' Enadenotucirev (EnAd) viruses into human trials following the successful treatment of cancer in clinically relevant mouse models of human disease. For all of the indications currently being investigated - bladder, lung, ovarian and colorectal – there is a huge unmet need to provide treatment options for those patients that do not respond to, or relapse following treatment with current standard of care therapies.	
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)?	EnAd is an oncolytic (cancer-killing) virus currently in clinical trials to treat a range of late-stage cancers. These clinical trials were supported by data generated under another project licence, work which will be built on under this licence. In these clinical trials, EnAd has demonstrated a good safety profile as well as successful delivery to disseminated tumours, and there are early indications that this may be associated with a favourable anti-tumour immune response. This project aims to assess new variants of the virus designed to offer improved anti-tumour efficacy through 'arming' to	

	produce therapeutic agents within the tumour to assist the immune system in identifying the tumour as a foreign body and destroying it. The most notable potential benefit would be the successful translation of an 'armed' EnAd virus into the clinic and its approval for the treatment of patients with diseases for which few or no other treatment options are available.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will use mice only, and will use fewer than 7500 mice over a 5 year period.
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?	All of the work carried out under this licence will be of moderate severity or below and the majority will be mild. The most common expected adverse effect will be tumour ulceration, whereby small wounds can develop on the surface of the tumour. Wherever this occurs mice will be killed by an approved humane method but appropriate samples will be taken post-mortem to ensure no animals are wasted. Weight loss and reduced mobility may occur in a small number of mice where tumour cells are delivered intravenously, both of which will require that the animal is killed to prevent suffering. Observations made during post-mortem analysis of killed mice will be used to refine models and tighten humane endpoints to ensure suffering is always minimised.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The transition of experiments into animal models follows on from extensive *in vitro* work to ensure each candidate virus is fully functional, and that the agent(s) with which the virus has been 'armed' are both expressed and functional in a range of *in vitro* experiments. This ensures that no animal work is carried out where the desired information could be obtained via alternative methods and that *in vivo* studies are designed around a deeper understanding of the 'armed' viruses being assessed. This limits the number of mice required under this licence but it cannot replace them entirely. Full evaluation of the virus candidates is not feasible *in vitro* as systemic delivery, interactions with the immune

	system, efficacy and toxicity are functions of complex interactions in a living system.
2. Reduction Explain how you will assure the use of minimum numbers of animals	• *In vitro* assessment of virus candidates in assays with stringent pass criteria ensures that animals are not used as a 'first pass' for determining if a particular batch of virus is functional. • Once we implant mice for a study, aiming to obtain a calculated number of tumours we design smaller studies to utilise tumours that come up either early or late such that no mice are 'wasted.' • Extensive planning and regular *in vivo* meetings within our research team ensure that we get the most out of each study. • Power calculations are carried out prior to embarking on studies, utilising data acquired in pilot studies and the wealth of data obtained in the last 8 years, to ensure that group sizes and weighting are appropriate to minimise the need for repeating experiments and to ensure that excessive numbers of mice are not used. • Studies using non-invasive imaging of both tumour growth and virus activity has enabled the measurement of outputs in real time without killing animals. This enables us to monitor the growth of 'internal' tumours such as those growing in the lung or liver, or the spread of viruses in real time, increasing the amount of useful information gained from each animal while decreasing animal numbers required.
3. 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.	All worked carried out under this project licence will utilise mice. Mice are the species with the lowest neurophysiological sensitivity in which our work can be carried out, and many transgenic mouse models are readily available and extensively studied which can be used to bridge the gap between mouse and human immunology. A great deal of care is taken to ensure that our studies are as refined as possible, to minimise suffering. The process of refinement runs in parallel to all of our *in vivo* studies to ensure that even 'tried and tested' models are subject to scrutiny, and to enable the application of more appropriate humane end points wherever possible. General procedural refinements applied to studies carried out under this project licence include: • Treatment will always be delivered at the smallest effective delivery volume.

	• Warm boxes will be used to maintain body temperature where extended anaesthesia is required and to dilate blood vessels for injection to minimise bruising. • Post-mortem analysis of mice at the end of studies enables us to better understand our models and to apply tighter humane endpoints wherever possible. • Human blood utilised in studies bridging the gap between human and mouse is obtained from clinical sites that allow for the same donors to be ordered repeatedly. This avoids toxicities associated with the immune cells from some donors adversely reacting with mouse tissues. • Animal welfare meetings held with other local licence holders enables the sharing of information so we can all ensure our studies are designed around a broad knowledge of current best practice.

Project 69	**Normal and leukemic blood cell development**	
Key Words (max. 5 words)	Stem cells, Infant leukaemia, Transplantation	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	1. To study the normal process by which fetal and adult stem cells produce mature blood cells especially red blood cells. Although adult blood cell production has been studied; not much is known about how blood cells develop in fetal life and this study will help address this question. 2. There is growing evidence that many childhood leukaemias start to develop in fetal life. Some rare leukaemias that develop very early (<12months of age), are called infant leukaemia and these children tend to do poorly even with intensive treatment. Newer treatment strategies are urgently needed for infant ALL. We would like to investigate the fetal cells that undergo leukaemic transformationin in infant ALL. Fetal progenitor cells will be modified by inducing the abnormal gene(s) to see if that is sufficient for leukaemic transformation. To unravel the processes leading to leukaemia in infancy/ childhood, we will either induce leukaemias in mice using these genetically modified human fetal cells or inject the mice with leukaemic cells from	

	patients.
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)?	There are a number of key areas where this research will have an impact on science and might improve treatment of patients with blood diseases: 1.To help understand the way blood cells mature from haematopoietic stem cells and mechanisms that control development of red blood cells. This in turn will inform us about childhood diseases that affect red blood cell production and how they can be managed. 2. To improve our understanding of therapy resistant infant leukaemia. This study will enable us to mimic blood cancers in mice and study in detail the disease pathways that may be targeted for novel therapies in infant leukaemia. These treatments will hopefully be more effective and less toxic than those currently available; and will lead to increased survival of infants with leukaemia.
What species and approximate numbers of animals do you expect to use over what period of time?	5000 mice over 5 years
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 protocols in this application are all of prospective moderate severity. The potential adverse events primarily relate to: • Immunosuppression and resulting infection • Irradiation and bone marrow transplantation • Leukaemia development • Administration of substances Welfare of animals at risk will be carefully and regularly checked. If some animals are in pain or exhibit other adverse effects, pain-killers, antibiotics or other treatments may be given under veterinary direction or humanely killed. Mouse strains showing any unexpected ill-health will be humanely killed. Most animals will be killed at the end of the protocol, in all cases ≤24 months of age. Tissue sample from these animals (blood, bone marrow, liver and spleen) will be examined for human cells and leukaemic

	disease burde. Human cells isolated from these tissues may be used for repeat transplantation assays; and/or *in vitro*/ molecular studies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Blood formation and leukaemia development are precisely controlled processes that require the living environment, such as bone marrow. HSCs are known to interact with other bone marrow cells, and when exposed to culture (non-living conditions), they change their properties. Therefore, these processes have to be investigated using animals. The mouse is the most widely used system to study the formation of normal blood and blood cancers. Mouse models have demonstrated to be highly relevant and essential for development of an understanding and clinical application of the blood forming system in man, not the least application of bone marrow transplantation and understanding of leukaemia since mouse and human stem cells share similar properties. Other advantages of the mouse model (apart from it being mammalian) include the availability of laboratory reagents to study blood functions. Furthermore, leukaemia models in mice can be used to understand the origins of the disease and to develop/ test potential targeted therapies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The laboratory has a number of systems in place to ensure minimal numbers of animals are used: • Limit cage numbers through weekly checks • Determining the use for animals in all cases prior to weaning • Use of appropriate number of animals in each experiment with careful experimental planning and statistical considerations to maximise the amount of information obtained from each animal e.g. serial blood sampling • Maximising yields of blood cells from each mouse for experimental use, for example, through optimal use of antibodies and nanofluidic molecular platforms developed in the laboratory allowing analysis of lower numbers of cells.
3. Refinement	When undertaking work with animals to achieve our

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.	aims we have carefully chosen the least severe procedures to minimise the pain and adverse effects for the animals. Specific examples of refined procedures in the laboratory include: *Conditioning of haematopoietic cell transplant recipients with split radiation dosage* In order to detect the activity of the transplanted cells, the host animal's own haematopoietic system must first be depleted by irradiation, in the same way as is done with humans receiving bone marrow transplantation as a therapeutic modality. In order to minimise the morbidity and mortality associated with irradiation, a split of two half doses of irradiation, rather than a single full dose which, in association with temperature and noise monitored housing, provision of moist food and extra bedding, and rigorous monitoring has resulted in very low levels of morbidity and mortality. Recepient mice will be given prophylactic antibiotics. *Housing of animals* We house all our animals in individually ventilated Cages (IVCs) which keep grouped animals separated from other animals and possible exposures, including exposure by air. Cages, food, water and bedding will be sterilised. *Training of PIL holders and our lab staff* To ensure that these protocols are carried out to the highest standard by competent, extensively experienced individuals, a rigid, formal process of training of all PIL holders and staff is are continued to be in place. *Administration routes* Cells, drugs and biologically active agents will be administrated by the least invasive route in the first instance when multiple routes are available for example *orally in water or feed rather than by intraperitoneal route. Procedures that are likely to*

	cause more than transient discomfort will be performed under anaesthesia with adequate cover with pre and post operative painkillers. Aseptic techniques will be used for administration of substances with special consideration to maintaining bosy temperature and hydration. If multiple subcutaneous injections are required, an implanted mini-pump will be used.

Project 70	Regeneration/neoplasia of nervous system and muscle
Key Words	stem cells, organ regeneration, brain tumours
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;

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

Brain tumours are the second leading cause of cancer related deaths in males ages 20-39 and the fifth leading cause of cancer-related deaths in women ages 20-39. Patients with glioblastoma survive about 140 days on average rising to 14 months if they are suitable for radical treatment. Survival trends for these patients have not improved in recent years and these tumours represent a rare exception to the general trend of cancer survival in the UK. Brain tumours also account for a large proportion of childhood tumours. Medulloblastoma is the commonest brain cancer seen in young children. The main treatments available to children with medulloblastoma today are surgery, radiotherapy or chemotherapy. These treatments can be effective and kill the tumour cells in a proportion of patients; however they are almost invariably also causing severe side effects which are particularly damaging in young children as their brain is growing quickly.

We are studying the cell of origin of brain tumours and we are trying to identify novel genes and pathways that control their behaviour. In particular we are looking at stem cells: these cells are found in every organ and can develop into specialised cells, thus contributing to the maintenance and ‘regeneration’ of the organ. We find cells behaving as stem cells in tumours too, they play an essential role in “regenerating” the tumours and so killing them is crucial. We are trying to establish the difference between these tumour stem cells that keep proliferating – producing other tumour cells endlessly – and normal stem cells, which stop proliferating when the body stops needing new cells. If we can understand how a tumour stem cell controls its

proliferation, we can start generating more targeted drugs that specifically kill these cells in a more effective and also much less toxic way.

We also trying to understand whether there are biological properties of tumours that can be safely exploited to enhance the regenerative properties of normal stem cells, in particular we are focussing on the stem cells of the muscle. The muscle is an organ with great potential for regeneration, however in many diseases the muscle lose the ability to regenerate. We want to understand whether the muscle can be supported in its regeneration by strengthening the stem cells.

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)?

This research will help us understanding how to improve the regenerative potential of adult organs and at the same time will help us understanding the origin of diseases linked to impairment of stem cells, such as for ex. brain tumours. If we know what allow tumours to growth, we will be able to design new more effective therapies. At the same time we may be able to manipulate basic mechanisms of stem cell functions to enhance regeneration of healthy organs.

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

We will use approximately 20000 wild type and genetically modified mice over 5 years. Whilst a proportion of these mice will develop symptoms of the diseases we want to study, the majority of them will only be used for breeding and/or for procedures which do not cause discomfort to the animals.

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?

Some of the animals will develop sign and symptoms related to the brain and muscular pathology induced incuding circling, tremor, disturbance of equilibrium, decrease in food intake, seizures and impairment of locomotion. The overall severity of these experiments is moderate.

Application of the 3Rs

Replacement

Mice are the animals best suitable for this type of experiment: They are vertebrates and they are mammals, so they share many of the physiological properties found in humans. The mouse, like humans, develops similar brain and neuromuscular structures and the presence of stem cells within these organs as well as the almost identical mechanisms involved in growth and differentiation of these cells makes the mouse brain and muscle ideal model systems to study diseases of these organs.

Reduction

We are constantly reviewing our procedures in order to improve wellbeing and to reduce the numbers of animals used. Where possible, we utilise approaches that do not require animals such as cell culture and computational biology. Unfortunately, it is not possible at the moment to completely replace the use of animals with other approaches.

All our experiments are planned on the basis of statistically robust sample size calculations to ensure the experimental groups are neither too large nor to small to detect the effects of the procedures. Control groups will be shared between studies whenever possible.

Refinement

All the experiments planned for this research build on a long tradition at this institution of treating animal welfare as a priority. Almost all of the experimental approaches to be adopted have been performed here many times in the past and are associated with detailed standard operating procedures aimed at minimising the number of mice to be used. When any new genetic lines of mice are generated, or if a substance is administered to an animal, they are examined very carefully for any signs of abnormality. We perform extensive literature searches to assess the continued validity of our models and to design, develop and implement measures which refine lesioning methods particularly in relation to lesion size and severity, We use anaesthesia and analgesia when necessary. We have recently introduced multimodality imaging, which allows examination of animals at multiple and repeated timepoints, importantly also at time when the animals are asymptomatic. This enables a timely identification of the lesions and also a reduction of the number of animals required.