Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 17

Projects with a primary purpose of: Basic Research – Oncology

Project Titles and keywords

1. **Analysing the genetic and epigenetic control of cancer**
    - Cancer, Genetics, Epigenetics

2. ***Helicobacter pylori* virulence, immunity and disease**
    - *Helicobacter pylori*; virulence factors; carcinogenesis; immunomodulation; allergy

3. **Models of intestinal & genetically related tumours**
    - Colorectal tumours, cancer evolution, genetics

4. **Radiation-induced leukaemogenesis**
    - Leukaemia, radio-sensitive, bone marrow

5. **Investigation of tumourigenic pathways**
    - Tumour, oncogene, signalling

6. **New Strategies for the Diagnosis and Treatment of Glioma**
    - Brain tumour, Glioma, Diagnosis, Treatment

7. **Glioblastoma treatment and resistance**
    - Cancer, brain, rodent, treatment, resistance

8. **In vivo models of childhood leukaemia**
    - Childhood leukaemia, new therapies

9. ***In vivo* studies of tumour associated fibroblasts**
    - Fibroblast, cancer, mouse, genetically modified

10. **Experimental cancer chemotherapy**
    - Xenograft chemotherapy non-genotoxic

11. **Arginine methylation and tumourigenesis**
    - Cancer, genetically engineered mice

12. **Epigenetics of Cell Senescence, Cancer and Ageing**
    - Epigenetics, Cancer, Ageing, Mouse models

13. **Analysis of stressors, nutrition and ageing in cancer**
    - Stress, cancer, treatment, ageing, diagnostics

14. **Tumour progression in Zebrafish models of cancer**
    - Cancer, Cell Signalling, Biosensor, Tumour Microenvironment, Optical Projection Tomography (OPT)

15. **Investigating Human Pancreatic Cancer in Mice**
    - Pancreatic cancer, genes, therapy

16. **Analysing cancer metastasis and therapy failure**
    - Cancer, Cell Movement, Imaging, Metastasis, Treatment

17. **Elucidating Novel Actionable Mutations in Cancer**
    - Enzymes, Genetics, Cancer Drivers, Drug Pathways, Tumour-Suppressing Enzymes

18. **Aspects of cancer regulation**
    - Cancer, Macrophage, dormancy, lncRNAs

19. **Cellular and molecular haematopoietic dynamics in health and disease**
    - Stem cells, haematopoiesis, transplantation, intravital microscopy, stem cell niche

20. **Imaging of Cell Therapy In Tumour Models**
    - Tumour, cell therapy, imaging

21. **Pancreatic cancer – improving our understanding and therapeutic options**
    - Pancreatic Cancer, Therapeutic, Genotype/Phenotype

22. **Targeting the mechanisms of tumour vascularisation**
    - Cancer, metastasis, tumour stroma, angiogenesis, therapy

23. **Defining mechanisms of cancer progression and dissemination**
    - Cancer, Metastasis, Microenvironment, Imaging

24. **Assessment of tumour initiation and metastatic spread**
    - Cancer, Metastasis, Drugs, Endocrine Resistance

25. **RUNX1 functions in normal tissues and cancers**
    - Haematopoiesis, leukaemia, cancer, reprogramming

26. **Cytokine signalling in development and disease**
    - TGF-13 superfamily signalling, transcription, cancer, embryonic development, signal transduction

27. **NKT cells and related immunity in health & disease**
    - Cancer obesity NKT (cells) immunotherapy

28. **Signalling pathways in cancer**
    - Cancer, protein kinase, protein phosphatise

29. **Mouse models of Prostate cancer initiation & progression**
    - Cancer, prostate, tumour-initiating cells, metastasis, therapy

30. **Characterisation of cellular heterogeneity in ovarian cancer**
    - Stem cells, xenotransplantation, cancer, lineage- tracing

31. **Mouse models of KRAS-driven cancer progression & therapy**
    - Lung cancer, oncogene, metastasis, therapy

32. **Inflammation and Viral Tumourigenesis**
    - EBV, cancer, chronic inflammation, anti-oxidant

33. **Mouse Models of Human Cancer**
    - Cancer, GEMs, therapy, transplantation

34. **Investigating epithelial cancer in vivo**
    - Colorectal Cancer, Pancreatic Cancer, therapy

35. **Cancer imaging and drug development**
    - Cancer, imaging, diagnosis, early response, drug development

36. **p53-family in physiology and cancer**
    - Cancer, aging, development, metabolism

37. **Novel functions of p53 mutations in tumour progression**
    - Tumours, mutant p53, RCP, engulfment

38. **Combining immunotherapy with current cancer treatments**
    - Cancer, oncolytic virus, magnetic particles, MRI, macrophages

39. **Validation of Tumour Vascular Targets and Associated Therapies**
    - Angiogenesis, cancer, vascular targets

40. **Models advancing knowledge and treatment of paediatric brain cancer**
    - Paediatric, brain, cancer, treatment, biology

41. Evaluation of novel drug formulations in pancreatic cancer

- Cancer, drugs, nanotechnology, imaging, targeting

Project 1	**Analysing the genetic and epigenetic control of cancer**	
Key Words (max. 5 words)	Cancer, Genetics, Epigenetics	
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)	Deaths related to cancer are the second leading cause of mortality in the UK, and the development of novel diagnostics and therapies remains an unmet clinical need. This project is aimed at increasing our fundamental understanding of the causes of cancer and the development of experimental preclinical models of human cancer that will accelerate the translation of novel therapies into the clinic. It is now clear that genetic status is fundamental to altered predisposition to cancer, cancer progression and response to therapy. Understanding the biological mechanisms associated with these genetic changes will be vital to facilitate new treatment strategies for human disease. In this project we wish to approach these need using existing and new mouse models of cancer available to achieve two primary goals. First, we wish to better understand the fundamental biological mechanisms underlying cancer. Second, we wish to explore how this knowledge might be used therapeutically, especially in the context of altered genetic predisposition.	
What are the potential benefits	This project aims to investigate the normal function of	

likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	genes implicated in tumour development and tumour suppression. The knowledge obtained will underpin future strategies for the development and use of novel therapies. This is particularly relevant to the treatment of cancer, as at present there are relatively few new options available in the clinic to modulate the course of disease. We furthermore hope to gain an understanding of the degree by which genetic status determines the response to a range of therapies. The majority of clinical benefits are likely to be long term; our principal hope is that by identifying candidate genes and candidate genetic pathways we will be able to refine and accelerate drug development for human therapy. There will also be short term benefits, primarily from the testing of novel drug therapies within our models. This latter approach has the potential to directly and immediately modify clinical practice.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse, approximately 43,000 over the five year course. This number is large partly because the group of workers and projects associated with this licence is large (at any one time, usually in excess of 30 people). The number is also large because of the complex genetics we use, which requires significant breeding programmes to create the correct combination of genes that we need. Over recent years we have consistently used in excess of 10,000 mice. As we diversify into new animal replacement strategies we actually foresee a reduction in animal numbers to less than 10,000 per annum.
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?	During the project, we will generate and analyse mice with both increased and reduced predisposition to cancer. This will involve the use of transgenic procedures to modify the genetic status of mice. The techniques we will use are such that we can control genetic status through exposure to chemicals or through exposure to viruses. We will also use these strains to model anti-cancer therapy in the clinic. This will be achieved by exposing mice to therapeutic agents and monitoring the response of normal and tumour tissues to these therapies. Monitoring will be performed both by live imaging mice and by

	analysing tissue samples at death. The adverse effects will therefore be the development of cancers and the negative side effects of anti-cancer therapies. In terms of severity, these will be mostly mild, but in some cases will be moderate. All animals will be killed at the experimental end point.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We aim to investigate the genetic control of cancer development and the responses of cancers to a range of agents including therapies. The extremely complex nature of such *in vivo* responses makes it impossible for these studies to be completely recreated in artificial systems in vitro. However, we have been pursuing the establishment of alternative procedures that have the potential to at least partially replace the use of animals. Most prominently we have been developing 3-dimensional culture systems for both normal tissues and tumour counterparts. To date we have established these for colorectal tissues and have begun to develop this approach for prostate and mammary tissues. Where possible and appropriate, we are using these *in vitro* approaches to inform our *in vivo* studies, with a view to replacing some in vivo studies and reducing and refining others, for example by establishing more precise hypotheses which can be directly tested in vivo.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our studies make use of the known available genetic models of disease and novel highly specific genetic models. By using such precise genetic models, we have enhanced the specificity of experiments permitting us to investigate precise genetic pathways and mechanisms. This reduces the overall numbers of animals required. We will also continue to introduce methodologies, such as novel imaging technologies, to increase the amount of data obtained from single animals and therefore reduce total animal requirement.
3. Refinement Explain the choice of species	The approaches we will use are fundamentally a refinement of using mice, as the creation of

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.	appropriate precise genetic models ensures that the work carried out will have the most accurate and useful outcome. We will also derive multiple endpoints and increase the amount of data generated from each animal, principally by the use of in vivo imaging to allow tumour progression to be monitored over time within the same animal - so reducing the requirement for multiple timepoints and thus animal usage. To minimise welfare costs to the animals we will monitor and examine animals on a daily basis and respond to any change in health status as appropriate. We will also adopt techniques wherever possible to minimise physical intervention. For example, we will maximise the use of genetic approaches that do not require chemical injections to induce the desired genetic change and we will use non-invasive approaches (such as imaging) wherever possible.

Project 2	**Helicobacter pylori virulence, immunity and disease**	
Key Words (max. 5 words)	**Helicobacter pylori; virulence factors; carcinogenesis; immunomodulation; allergy**	
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
		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 aim of this project is to determine the mechanisms by which certain factors from the human bacterial pathogen *Helicobacter pylori* affect the host response, and lead to gastric cancer development. We wish to utilise this information in designing strategies for manipulating the immune response to infection, in order to avoid gastric cancer outcomes. The secondary aim of this project will determine how the immune response to *H. pylori* protects against allergy, and develop bacterial components as putative therapeutic agents for allergy and asthma.	
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 is a great need for new therapeutic strategies to treat and prevent gastric cancer as the 5 year survival rate in the UK is only 17%. The study will determine how the cellular immune response contributes to and protects from gastric cancer, and the influence of *H. pylori* strain differences. We aim to develop a strategy to reduce gastric inflammation and thus inhibit cancer development. The work has	

	important implications for the management of *H. pylori* infection, and may inform more broadly on the relationship between immunity and cancer development at other sites in the body. The project will also help in understanding how *H. pylori* infection protects against allergic diseases such as asthma. The prevalence of these conditions has increased dramatically over the past 30 years. Understanding mechanisms behind the protective effect of *H. pylori* infection, and the bacterial factors that drive this response may lead to future advances in the development of therapeutic agents. We regularly present our data at international conferences, and have been successful in publishing our work in high impact scientific journals. We will continue to do this to aid in the advancement of this important area of medical research.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice – 1400 over 5 years Mongolian gerbils – 480 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?	*H. pylori* infections do not usually result in adverse effects, however long-term colonisation can result in weight loss and the development of gastric tumours. The house dust mite allergy model is mild and does not usually result in adverse effects. Administration of allergens, immunomodulatory substances, purified *H. pylori* components or suppressive immune cells may rarely cause slightly increased susceptibility to infections, respiratory symptoms or anaphylaxis, or cumulative toxicity from repeated exposure to inhaled anaesthetic agent. The expected level of severity for these adverse effects is Moderate. Animals will be killed using a Schedule 1 method at the end of the experiment.
Application of the 3Rs	
1. Replacement State why you need to use	Human epithelial cells from the stomach lining can only be cultured for very short periods *in vitro,* and the cell lines currently available are derived from

animals and why you cannot use non-animal alternatives	cancer tissues. As they are already tumour cells, this makes them unsuitable for understanding events that lead to cancer development. The immune cells present in mucosal tissues differ greatly from those in blood. In order to study the gastric mucosal immune response to the infection, and how it influences the development and course of allergic disease, we require intact gastrointestinal and respiratory systems. This can only be achieved using whole animals or humans. We cannot test the effects of experimental immune modulating substances on humans at early stages in the research, therefore we must use animal models. Animals will only be used in experiments where it is not possible to perform studies by other means. Hypotheses will be developed using observational data from human studies before performing experiments on animals to ensure that relevant parameters are being measured.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The mouse and gerbil models for *H. pylori* infection and the mouse allergy model are well-characterised in the literature and we have a great deal of experience with them. As inoculation doses, timings and the number of weeks necessary to observe immune responses and pathology are therefore already known, will avoid optimisation experiments. For the majority of mouse studies, inbred strains will be used in order to reduce the number of animals required per group with statistically significant results. Outbred gerbils will necessitate larger group sizes. Experiments will be carried out according to randomised block designs and statistical analysis carried out using non-parametric tests. The Mann-Whitney U-test will be used to determine differences between sets of independent data from two treatment groups, and the Kruksal-Wallis test for equality of medians will be used to compare the results from multiple groups. The minimum group sizes required to obtain statistically significant differences have been calculated using power equations on previous experimental data. For a power of at least 80%, it

	was calculated that group sizes should be of 6-8 mice and 8 gerbils when comparing colonisation densities of *H. pylori* strains. For experiments comparing immune responses to different strains, group sizes are likely to require 8 mice and 11 gerbils. With the block design, experiments will be designed to test alternative infections and treatments in small batches of 3-5 per group, building up to a maximum of 12 mice or 20 gerbils per group. Short-term experiments are likely to provide statistically significant data with groups of 6, as found previously.
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 architecture of mammals is required to accurately model the results of human infection. Mice and gerbils are of the lowest neurophysiological sensitivity yet retaining similar physiological characteristics to humans. They are well-characterised models for *H. pylori* research, with similar immune responses to humans. We have previous experience with the Der p 1 allergy model and we continue to work with allergy experts to ensure that our techniques are as refined as possible. The gerbil is the best model for *H. pylori*-induced gastric carcinogenesis. These animals can be colonised by virulent strains, which induce similar pathology as in humans. Well-established and refined procedures will be used. We will monitor chronic *H. pylori* colonization non-invasively using commercial stool antigen tests. Repeated allergen delivery is necessary to ensure sensitisation of all animals. Where the aim of an experiment is to measure the immune response to infection, the severity limit will usually be Mild. Where the aim of an experiment is to study carcinogenesis, however, animals will need to be infected for longer periods in order for pathology to develop. The severity limit for these experiments will be Moderate (weight-loss <15%). Changes in the body mass of animals will be followed by weekly weighing.

Project 3	**Models of intestinal & genetically related tumours**	
Key Words (max. 5 words)	Colorectal tumours, cancer evolution, genetics	
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)	Cancers grow because their genes are in some way altered or faulty. An increasing number of faulty genes that are important in causing cancer are being identified but we often do not know how those faults lead to cancer growth. In addition, we know that cancers have a lot of faulty genes that have little or no importance in their growth, but it can be very hard to tell which are the functionally important genes. Without that knowledge, we cannot target the right genes when we treat cancer. This project aims to examine genes that are faulty in human cancers and (i) work out whether they are important for cancer growth, (ii) work out how the faults lead to cancer and (iii) assess treatments that might be effective against cancers that carry those genetic defects. We also wish to look at how cancers change as they grow and acquire more genetic changes, as we know very little about this important process because it cannot easily be studied in humans. Our focus is on colorectal (bowel) cancer, which is one of the most common cancers and is often hard to treat. We aim to make mice that have human-like colorectal cancers or benign tumours that can turn into cancer. We shall also look at a few other types of cancer that share some of	

	the same faulty genes with colorectal 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)?	We hope that we can obtain fundamental knowledge about how cancers grow. For example, if we treat cancer with a particular drug, all the cancer cells may be killed or some or all of them may be resistant to the drug. By looking in great detail how the changes caused by the drug come about, we can, for example, design new drugs or combinations of drugs that could be more effective than current ones. Another major part of our work will work out how the gene changes actually make the cells that form a cancer different from their normal counterparts. By doing this, we may spot weak points that can be attacked by new drugs to try to cure cancers, or that can be used to prevent cancers, especially for the people at highest risk.
What species and approximate numbers of animals do you expect to use over what period of time?	We plan to use about 20,000 mice over 5 years. A large proportion of these animals will be used for breeding. The reason for this is that we want to re-create the complex genetic changes that occur in human cancers by making combinations of several faulty genes. This provides the best way of mimicking human colorectal cancer and is important for things like finding out whether a cancer is resistant to a particular drug.
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 of the mice will develop benign tumours or cancers and these will usually cause predictable symptoms such as weight loss and general ill health. However, in the great majority of cases we can identify those cancers before they cause the animal any distress and, after humanely killing the animal, we can gain enough information to answer the questions we are asking. For some mice in which the timing of cancer development is uncertain, we will regularly monitor for evidence of tumours and intervene early to prevent distress. We may also have to give some mice injections, but that will only cause temporary discomfort. Overall, we expect most of our mice to have disease of only mild severity, with a small proportion having disease of moderate severity. At the end of the work, all 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	Our work is all derived from studies of human cancer patients. However, there are good reasons why we cannot obtain all the information we need from humans. For example, we cannot simply leave cancers in the body and examine how they develop, we cannot modify genes or gene combinations in humans, and we cannot test unproven therapies in humans. In other cases, some human diseases are too rare to study in patients. We can do some of these experiments in cell lines derived from human cancers, but these lack certain very important features. We know that the interactions between cancer cells and normal cells are vital determinants of patient prognosis and how well the cancer responds to treatment, and these interactions are very hard to model realistically in cell lines Only animal models can faithfully mimic a human cancer and, even if not every aspect of tumour growth is the same in humans and mice, there are many similarities. For example mice carrying mutations in genes that predispose to bowel cancer in humans also develop multiple bowel tumours that have provided many important insights into the human disease
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our studies are based on the minimum number of mice required to show differences between groups, such as response to a new drug or the effect of a specific gene fault. Where possible, we perform initial studies in cell lines or other cell culture systems before turning to mouse models.
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.	Compared with other animals, mice currently provide the only model of human disease that allows routine genetic manipulation, that has sufficient physiological similarity to humans, that comprises a large body of existing resources, and that can be bred with sufficient rapidity. The existing data on likely phenotypes and dosing of mice are far more comprehensive than those for other animals, preventing unnecessary animal use. Even when we cannot be certain of what tumours, if any, a mouse will develop, there are precedents that will allow us to anticipate when phenotypes will develop and whether they could cause the animal any distress. If necessary, pilot experiments will be perform on small numbers of mice in order to determine windows where

	research can be performed successfully before distress develops. All animals will be monitored regularly – daily in the majority of cases – and any mouse with evidence of distress will be humanely killed. Wherever possible, we shall humanely kill mice before they show any signs of distress. We shall further refine our experiments using specific techniques, including - use of anaesthesia for any procedure expected to cause more than momentary distress - using targeted and/or inducible mutations so as to minimise ill effects outside the window of study - minimising the delivery of agents to promote or retard cancer growth and using non-invasive methods wherever possible - using modern methods of gene induction that require fewer breeding steps

Project 4	**Radiation-induced leukaemogenesis**	
Key Words (max. 5 words)	Leukaemia, radio-sensitive, bone marrow	
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 objective of this project is to obtain mechanistic and quantitative data on events that lead to radiation-induced acute myeloid leukaemia (rAML) appropriate to developing and refining risk projection models in humans, particularly at low doses. To do this we will use an rAML-sensitive mouse strain which has been genetically modified to carry a fluorescent “reporter” gene that can be used to identify characteristic rAML-associated chromosome lesions in target cells at early timepoints post-irradiation. The appearance of these characteristic lesions will be followed over lifespan using regular blood sampling. This will allow the timing of key events in leukaemogenesis to be identified. We will also use these strains to investigate the role of target cell/stromal interactions in leukaemogenesis, by isolating haematopoietic stem cells and progenitors from irradiated donor mice and using them for transplantation assays in to AML-sensitive and non-sensitive host strains. In addition we will investigate the effects of radiation	

	treatments on the expression of genes indicative of radiation exposure or those potentially involved in acute myeloid leukaemogenesis. We have developed sensitive techniques for gene and protein expression analysis to identify radiation-induced changes to gene and protein function.
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)?	All the information from the project would feed into the development of refined models for cancer risk estimation in human populations, particularly with respect to low dose radiation exposures. Current risk estimates have 9000 cancer cases per year attributable to ionising radiation exposure in the UK. Of these, more than 1000 are attributable to artificial radiation sources. Many assumptions are made in these calculations either over- or under- estimation is possible due to the uncertainty on the true risks following from low dose exposures. Identifying and quantifying key events in radiation-induced leukaemogenesis is vital for refining these models. In addition, a greater basic knowledge of radiation induced carcinogenesis will be obtained and in the longer term this work will aid the identification of early markers of radiation exposure and associated disease. All studies that yield sound results will be submitted for publication in the open scientific literature and thus add to the knowledge base available to researchers worldwide. For example, the primary output of the current project will be scientific evidence in the form of peer reviewed scientific papers. This evidence will be consolidated through dissemination of project results and their implications to radiation protection specialists at the stakeholder meeting in the final year of the project.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice. Approximately 3500-4000 mice will be used over a five-year period.
In the context of what you propose to do to the animals,	During lifespan studies mice would be expected to develop Leukaemias and other cancers associated

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	with radiation exposure. Twice-daily checking by trained staff and use of a welfare sheet developed after extensive experience with these types of experiment mean that virtually all of these mice are humanely killed as early as possible when symptoms are identified. The level of severity is moderate. In bone marrow transplantation studies mice are vulnerable to infection and the transplant failing. Extensive measures are in place to monitor animals undergoing such procedures, and to prevent infection and provide extra nutritional and fluid support. If, despite these measures, a mouse begins to show symptoms of infection or failure of the transplant, they are removed from the study and humanely killed. The severity limit for this procedure is moderate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have to use animals for this project as it is not possible to recreate the complex process of leukaemogenesis *in vitro* using tissue culture. Haematopoietic stem cells (the cell of origin of radiation-induced acute myeloid leukaemia) cannot be maintained for more than a few weeks in tissue culture, and also we wish to investigate the effect of the microenvironment on the process of leukaemogenesis which is impossible to recreate in single cell suspensions or colony assays *in vitro*. In addition, human samples of radiation-induced AML are difficult to obtain both for ethical reasons and because of the relative rarity of these tumours in the general population. Even if samples were available it can be difficult to identify the exact exposure dose an individual may have received, and these samples cannot be used for mechanistic studies to identify early pre-leukaemic events.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our experiments typically involve batches of mice exposed to graded doses of X-rays. Batch sizes will be determined by statistical analysis techniques following advice from qualified and experienced statisticians available in house. This means that numbers of mice used will be the minimum required

	to give sufficient statistical power to each experiment. Also from lifespan studies multiple tissues and preparations will be prepared from individual animals thus increasing the data available from each experiment. Tissues, or materials extracted from tissues, will be stored for future use where appropriate. Both male and female mice can be used as a source of bone marrow cells and for early event studies, thus reducing the number of mice needing to be bred.
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 model of rAML we use is well established and has been used by laboratories worldwide. We have developed a scientific understanding of this model system that allows us to design experiments that produce the maximum amount of data in the minimum number of animals, and also have substantial experience in the experimental techniques required. In addition, the extensive amount of genetic and proteomic data available for the mouse make this organism amongst the best model for human disease. We will minimise animal suffering by identifying potential adverse effects and ensuring that humane endpoints are developed and applied under these circumstances. We have developed welfare sheets and will ensure that staff involved in the day-to-day care of the animals are trained in using them. Where indicated by the protocol, animals will be housed in isolators with sterile food, water and bedding, to reduce the chance of advantageous infection.

<table>
<tr><td>Project 5</td><td>Investigation of tumourigenic pathways</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Tumour, oncogene, signalling</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></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>
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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>Our interests lie in the molecular processes that underpin the initiation, progression and maintenance of cancers. Our goal is to identify key pathways that drive and maintain tumours and to assess them therapeutically using mouse cancer models.

In particular we are interested in the pivotal roles of genes that either promote (oncogenes) or inhibit (tumour suppressors) cancer. Given that tumours form and are maintained by aberrant physiological processes we will also address their roles in normal tissue homeostasis and regeneration. We aim to generate novel mouse models that can be used to explore the critical functions of these and other oncogenes and tumour suppressor genes.

We previously demonstrated that systemic inhibition of Myc constitutes an effective and specific anti-tumour therapy in the absence of prolonged adverse effects on normal tissues. Further work is needed to assess the general applicability of these observations to other cancer types and to refine the Myc inhibitor to a</td></tr>
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	minimal region that may be mimicked pharmacologically. We aim to analyse the mechanism of tumour regression in these animals. We are now attempting a similar approach to test the therapeutic value of inhibiting other key tumour-promoting proteins. Using our mutant mouse strains we demonstrated that reinstating the function of the p53 tumour suppressor increases survival of mice with lymphoma. We aim to explore the contribution of p53 and other tumour suppressors to tumour suppression and how tumours evade such surveillance. Our studies cannot be conducted *in vitro* because tissue culture cannot recapitulate the complex cell interactions necessary for the development and maintenance of tumours in an animal. Growing cells in an unnatural environment outside the body activates p53 thus preventing study of tumour suppressor function. Nonetheless, we can learn a great deal from studying cells derived from these mice and such techniques will be employed wherever possible. The transgenes and genetic mutants we are interested in have only been generated in mice and no other species is suitable for our studies. Pilot experiments using small numbers of mice are performed to refine and inform subsequent experiments to use the minimum number of animals (approximately 20 per experiment) necessary for statistically significant information. Mice will develop tumours in various tissues either spontaneously or following administration of a gene-inducing/deleting agent. Normal and tumour tissue will be studied in parallel and tumour regression induced by gene activation/deletion or other agents.
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 focuses on oncogenes and tumour suppressors that are most frequently mutated in a broad range of human tumours. Although the involvement of many of these pathways in cancer is established, targeted therapies for many of the proteins involved do not exist - indeed, many of these targets are regarded as ‘undruggable’. We will test the

	idea that there are a small number of genes that funnel the activities of other oncogenes and that are essential for tumour formation and maintenance. Understanding how these genes work and whether their inhibition in mouse models has therapeutic potential will have broad application in advancing treatment of many, perhaps most, human cancers. One limitation of such inhibitors is potential to cause collateral damage to normal tissues – a possibility that can easily be addressed in our switchable mice. We have already developed and validated some of the mouse models that will be used in these studies. Additional genetically modified mouse strains will be developed and used in conjunction with existing strains and those available to us from commercial sources or from collaborators. Given our proven track record in the use of novel switchable mouse genetics we are highly likely to generate significant results. Our results will be published in influential peer-reviewed journals and disseminated through scientific seminars and mouse strains made available to the scientific community. They will also provide an excellent resource for testing potential new therapies by the pharmaceutical industry. The proposed programme will generate unique, novel and valuable data concerning the roles of oncogenes and tumour suppressor genes in tumour formation, maintenance and regression and are aimed at developing new, effective and specific ways to treat or prevent cancer in humans.
What species and approximate numbers of animals do you expect to use over what period of time?	We will only use mice during this study. During the next 5 years we anticipate that we will use up to 59550 mice, nearly half of which are required solely for breeding to generate multiple genetic modifications in sufficient individual animals for scientific significance.
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	Adverse effects will be minimized by using the earliest possible endpoints for tumour studies (not death). Approximately 85% of the animals are either sub-threshold or only reach a mild severity (slight or transitory suffering). Approximately 10-12% of the animals experience moderate severity, such as

happen to the animals at the end?	discomfort and abnormal behaviour that does not prevent eating, drinking and other normal activities). However, because of the variable nature of some cancer models, disease may advance rapidly in a cryptic manner with little or no previous clinical signs, so rarely animals may reach severe adverse effects for short periods before they are killed. Less than 0.5% of animals die because of tumours and less than 3% reach the severe stage (exhibiting one or more of the following: reduced activity with a hunched posture, piloerection, possibly tremors or fitting, dyspnoea, jaundice, neurological signs, digestive disturbance). Mice are carefully monitored so as not to exceed these endpoints. 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	Our studies address the role of tumour suppressors and oncogenes in the development, progression, maintenance and reversion of tumours. Such analyses cannot be conducted in vitro because tissue culture does not recapitulate the complex interactions necessary for the development and maintenance of tumours. Furthermore, tissue culture is carried out in an unphysiological environment (eg high continuous levels of cytokines, inappropriate oxygen tensions and high levels of ultraviolet light), all events that may activate p53 and select for unphysiological attributes. Neither can we use computer models, since too many ill-defined or unknown variables affect the expression of these genes. Since our overarching purpose is to demonstrate whether oncogene inhibition and/or reactivation of tumour suppressors elicits a valuable therapeutic index we have no alternative but to build in vivo animal models in which tumour arise, evolve and spread in intact tissues. Nonetheless, we can learn a great deal about cell-intrinsic pathways from in vitro studies of cells derived from these mice. In addition we are pursuing new technologies that allow genetic recombination in cultured adult mammalian cells in order to study these cell-intrinsic pathways.

2. Reduction Explain how you will assure the use of minimum numbers of animals	Pilot experiments using a small number of animals will be used to refine the experimental procedure and design. We then use the minimum number of animals required for statistically significant information — determined in collaboration with biostatisticians familiar with mouse models. Our switchable models give far better reproducibility than do sporadic models of tumourigenesis, further reducing the numbers of animals needed for statistical validity. Where possible we generate cell lines from these animals for in vitro experiments to guide our in vivo mouse work. Tissue from experimental animals will address more than one question. Control animals will be used for more than one study. Animal models incorporating fluorochromatic, bioluminescent markers or other imaging modalities allows longitudinal analyses of fewer animals. Finally, supernumerary wild type mice from our breeding strategies will be used as embryo recipients or vasectomized maLes. Genetically engineered mouse strains no longer required will be cryo-preserved by sperm and/or embryo freezing.
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 transgenes we are interested in have only been generated in mice. Our switchable models generate animals that exhibit no phenotype in the absence of the inducing agent but reproducible effects with agent (a significant refinement on sporadic models) and allows analysis of the immediate and long term consequences of each perturbation. We always select the earliest possible endpoint and routinely monitor mice to ensure that they do not pass these endpoints. Where possible we will use non-invasive imaging modalities to monitor and follow early stage malignancy. Where possible we will use the least invasive method of agent/drug delivery. For example, we are developing delivery methods via food and water and have successfully demonstrated the administration of tamoxifen in the diet thus reducing the need for

	repeated intraperitoneal injection. Likewise, we seek to restrict our studies of wound healing to moderate tissue damage — sufficient only to determine the role of oncogenic and tumour suppressor pathways in subsequent wound healing. The doses, kinetics and effects of the chosen agents chosen are well characterised. Nonetheless, for each agent we will carry out pilot experiments to ensure that the doses only cause minimum suffering.

<table>
<tr><td>Project 6</td><td>New Strategies for the Diagnosis and Treatment of Glioma</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Brain tumour, Glioma, Diagnosis, Treatment</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></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></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>A glioma is a brain tumour that arises from non-neuronal cells within the brain and accounts for approximately 45% of all primary brain tumours. The most malignant grade of glioma; glioblastoma multiforme, has very poor prognosis even after aggressive treatment of maximal surgical resection and chemotherapy with, most patients surviving only 12-15 months following diagnosis. There is therefore a need to develop new drugs or to deliver the drugs specifically to the tumour so less drug is needed and the unwanted side effects can be avoided. The aim of this project is to establish a mouse model of glioma so new drugs against glioma and strategies to deliver the drug directly to the glioma can be investigated. An important factor in favourable outcome for the patient is the time taken to diagnosis. Having a simple blood test for chemicals that are released by the cancer in to the blood would speed up diagnosis significantly. This project will also use the animals to generate blood samples so the profile of molecules circulating in the</td></tr>
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	blood can be characterised and related to type and size of tumour.
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 have already developed a number of new drugs that stop glioma cells from growing and novel aptamers (chemical antibodies) that bind selectively to glioma cells and glioma tissue from patient biopsies. By attaching them to established or new drugs for the aptamer will be able to deliver the drug directly to the glioma. This would reduce the amount of drug needed and significantly improve the side effects profile endured by patients. We have also identified biomarkers circulating in the blood of clinical patients. Having a full profile of the expression of these circulating biomarkers would significantly aid detection, diagnosis and monitoring of gliomas. As part of this project, we intend to assess the biomarker profile using blood/serum samples of the animal as a means to evaluate disease progression and predict response to treatment.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 1000 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 project will implant tumour cells into the brain or flank of mice lacking an immune system. As the tumour grows, the size will be monitored by a non-invasive bioimaging system. or callipers for tumours growing under the skin of the flank. Some animals will receive drugs to try and reduce tumour growth Adverse effects could arise from the tumour itself or from the novel drugs being administered. The effects of tumour growth on behaviour will be closely monitored and severity limits will be kept to a minimum. Toxicity of novel drugs will be assessed in mice without tumours in the first instance prior to being in animals with glioma. Any toxic effects seen would necessitate culling the animal and a lower dose being used. Regardless all animals will be humanely culled at the end of the protocol and tissues will be used in other ongoing in vitro studies, eg histology, drug binding

	assays.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Any new drug needs to demonstrate it works in a model that replicates the clinical condition as closely as possible before the drug can be of use in the clinic. Unlike other tumours, brain tumours are removed from the rest of circulation by the blood brain barrier (BBB), and this poses significant complications when assessing new drugs which target the brain. *In vitro* BBB models do exist, and all of our agents will be tested in these models, but at present they do not fully mimic the complexity of the BBB in the live animal. Therefore, the only way to definitively assess the efficacy of our novel therapeutic agents is in an animal model.
2. Reduction Explain how you will assure the use of minimum numbers of animals	This licence application is largely to assess the effectiveness of new drugs that haven't been looked at previously, consequently it is not possible to determine the exact sample size as background data does not exist. Pilot studies using low numbers of animals will be undertaken in all cases with data analysed to determine for subsequent studies the minimum number of animals required to generate meaningful data to ensure only the minimum number of animals will be used. Experiment and drug trials will be designed, coordinated and appropriately analysed to minimise animal numbers. One example could be where two drugs having the same vehicle will be tested simultaneously so only one control group will be needed. Animals will be used to satisfy multiple purposes wherever possible so blood samples taken from mice with glioma used as a control in a study to test drug efficacy will be split where possible to determine levels of multiple biomarkers of interest.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having	Mice will be used as they allow the growth of a range of tumour models which mimic the clinical situation to a high degree, and are the species with the lowest neurophysiologic sensitivity in which such well-characterised models of cancer exist. Further there is a

regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	wealth of correlative studies between mouse and human that indicate that the results gained by their use will be translatable. In addition, procedures for the housing and care of both immunocompetent and immunodeficient mice are well established and their small size readily allows whole-body imaging. The use of non-invasive bioimaging refines the proposed project plan as this enables longitudinal studies to assess an individual animal at a number of different time points and reduces the number of animals that need to be used in the methods. Animal suffering will be minimised by implementing a score chart that measures well-being. Depending on the nature of the drug to be tested, it may be preferable to implant osmotic mini pumps to reduce the number of injections to the animal at later stages. Mice will be monitored on a daily basis and any animal that shows signs of adverse or unexpected responses, depending on the severity, either advice will be sought from the local NACWO and/or NVS or the mouse will be culled immediately to limit any additional discomfort**.**

<table>
<tr><td>Project 7</td><td colspan="4">Glioblastoma treatment and resistance</td></tr>
<tr><td>Key Words (max. 5)</td><td colspan="4">Cancer, brain, rodent, treatment, resistance</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="4">5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td colspan="4"><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 colspan="4">The aim of this project is to develop models of brain cancer using tumour cells from patients and use them to study how tumour growth and response to treatment varies between patients and over time.

<u>Can patient-derived animal models tell us how patients will respond to treatment?</u>

We will use the data of how animals respond to treatment and compare it with what happens to the patients then examine the clinical data to see if the models can in fact predict patient response.

These data will tell us if we can use these models to test drug combinations to select those suitable for use in clinical trials.

<u>Can patient-derived animal models tell us how patients become resistant to treatment?</u>

We will use tumours from in treated and untreated animals to identify the cells that dominate and drive</td></tr>
</table>

	treatment resistance. Examination of the genetics changes in these resistant cells will help us to identify new therapeutic targets in patients. We can then go back to our animal models to test new treatments to identify those most appropriate to evaluate in clinical trials.
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 use patient derived cell lines to generate mouse models of glioblastoma, which are more representative of the human disease than we currently have. We will use these models to study how tumour growth and response to therapy varies between patients and over time. These data will inform how we can place patients into groups and more accurately target the right drug to the right patient. We will use the models to investigate how tumours evolve in treated and untreated animals. This will help us to understand how minor cell populations can result in recurrent and treatment-resistant disease. By analysing the mechanisms of this resistance we will be able to identify ways to circumvent the resistance and improve patient survival.
W hat species and approximate numbers of animals do you expect to use over what period of time?	Mice Up to 3070 mice over 5 years Rats Up to 2750 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 level of severity? What will happen to the animals at the end?	Tumour burden will be limited to the minimum required for a valid scientific outcome. In all cases, the general health and condition of an animal will remain the overriding determinant, in accordance with the NCRI Guidelines for the Welfare and Use of Animals in Cancer Research. Most animals on protocol 19b1 (~70%) are expected to exhibit clinical signs of mild severity. Less than 5% of these will need to be humanely killed to ensure the moderate severity limit is not breached. General adverse signs may include • Weight loss (<20% of pre-op weight) or rapid weight loss (>10% pre-op weight in less than 24 hours)

	• Poor feeding or drinking (<24 hours post-op) • Wound infection (<1% of animals) Signs associated with tumour growth may include: • persistent weight-loss >15%; • food & water intake <40% of normal for more than 72 hours • limb weakness or reduced mobility • A single generalised seizures lasting >2 minutes. At the end of the experiments animal 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	Cells in a dish are unable to recapitulate the complex signals driving tumour growth. To evaluate treatment response demonstration of effects in animals is essential. Analysis of how animals process a drug is vital to inform the design of early phase clinical trials (fore example dosing schedules). We aim to use our models to help us make decisions about how to treat patients so the data we generate must be as robust as possible.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The use of inbred strains will be considered wherever possible in order to minimise variability and where possible transgenic animals designed to address specific questions will be used. This will use smaller numbers of animals compared to crude non-transgenic alternatives. Statistical analysis will be performed to ensure the number of animals used is the minimum necessary to generate a valid result. In vitro and in silico studies will be used where appropriate and the data used to design studies which use the least number of 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	The use of models using cells derived from individual patients represents a step-change in refinement. These animals are a more accurate re-capitulation of the human condition. Hence, fewer animals are required in many biological studies to obtain a given effect or to look for a biomarker.

general measures you will take to minimise welfare costs (harms) to the animals.	There is also growing evidence that cells from an individual GB patient will generate an in vivo model that retains the molecular & genetic features of the patient from whom the cells came. The programme will also use mice (or rats) in order to take advantage of genetically modified animals that can reduce the number of animals used in an experiment (e.g. use of immunocompromised mice to avoid repeated injection of immunosuppressant drugs). Pilot studies will be used to both refine the techniques used in each experiment and to minimise the number of animals used.

Project 8	**In vivo models of childhood leukaemia**	
Key Words (max. 5 words)	Childhood leukaemia, new therapies	
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 purpose of this project is to understand how abnormal cancer genes cause leukaemia and other blood diseases in children, and to use this knowledge to develop new therapies against these diseases.	
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 of this work will be the development of specific therapies for childhood blood diseases, such as leukaemia, which may be more effective than current therapies and which may have fewer toxic side-effects.	
What species and approximate numbers of animals do you expect to use over what period of time?	It is estimated that 5000 mice will be used over the course 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 level of severity? What will happen to the animals at the end?	All mice undergoing experiments will be closely monitored and any showing unacceptable signs of discomfort or disease will be humanely killed. All mice will be killed at the end of the protocols, or transferred to other project licences. Most mice will not show any signs of illness in this project. However, some may show clinical symptoms, such as weight loss (up to 20%), abnormal coat, segmentation of vertebral column, readily palpable dorsal pelvic bones and subdued behaviour patterns.	

	This will mostly be a result of leukaemia progression in transplantation recipients. Any animals showing one of these symptoms will be monitored daily and if there is no improvement after one week, they will either be sacrificed or the named veterinary surgeon will be consulted. Any animals showing more than one of these symptoms will be monitored daily and if there are no signs of improvement after two days, they will either be sacrificed by a schedule I method or the named veterinary surgeon will be consulted. In a small number of cases, some mice may die without showing any prior clinical adverse symptoms.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We plan to make use of extensive laboratory models to investigate how abnormal genes cause leukaemia and how leukaemia cells can be eliminated by new treatments. Although these experiments are important, they are limited in how closely they can reproduce disease occurring in patients. For example, many mutant genes have been shown to make normal blood cells immortal when grown on plastic in the lab. In these experiments, whereas normal blood cells can grow for a few weeks, the ones harbouring the genetic abnormality can grow indefinitely. However, in some cases when these cells are grown in experimental animals, they do not induce disease. This means that the genetic mutations cannot by themselves induce disease, but probably require additional mutations. This information can then be used to decide on how important the original genetic mutations is for the disease and likely novel therapies targeting this mutation are to succeed. There are many reasons for discrepancies between experiments performed in tissue culture and those modelled using animals. An important difference is the precise environment in the bone marrow where blood cells develop. This can have profound influences on disease progression, something which cannot be accurately modelled using tissue culture. Furthermore, novel drug treatments developed to specifically target particular genetic mutations may be very effective in tissue culture experiments, but this does not mean that they will work in people. For example, the drugs may be rapidly eliminated from the body whereas they may be stable in medium used in tissue culture, and some drugs may not be absorbed sufficiently well in the body for them to be effective at curing disease. To test the effectiveness

	of such drugs, animal models have to be used.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of mice used in this project will be kept to a minimum. Experiments in mice will only be performed if there is good evidence from laboratory experiments that the work in mice is likely to succeed. For example, new therapies will only be tested in mice once they have been shown to be effective at killing leukaemia cells in the laboratory. Wherever possible, we will use new techniques to minimize the numbers of mice used. For example, leukaemia bio-imaging will be performed as often as possible, which will reduce the number of mice necessary to identify an effective new therapy.
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 project will only use mice as the experimental animal. These are the most suitable animal to use because of the large body of experimental data and the current knowledge regarding blood development and blood diseases in mice. These processes are comparable to those in human beings in many ways. Significantly, a recent study has shown the sensitivity or resistance of particular forms of human leukaemia to chemotherapeutic drugs is accurately recapitulated in mouse models of the diseases in question. This makes such models particularly useful in testing novel therapies for these diseases. All mice undergoing experiments will be closely monitored and any showing unacceptable siqns of discomfort or disease will be humanely killed. In all cases, the least invasive and harmful experimental procedures will be used.

Project 9	***In vivo* studies of tumour associated fibroblasts**	
Key Words (max. 5 words)	Fibroblast, cancer, mouse, genetically modified	
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 biology is proving increasingly complex, with multiple host cell types recruited to tumours in addition to the genetically altered cancer cells. In addition, tumours have a significant impact on the function of distant organs and tissues, a process that contributes to metastasis. One type of host cell that is pivotal to cancer development and metastasis are fibroblasts, cells that produce and remodel connective tissue including collagen. Fibroblasts are now known to be several cell types that be recruited from sites such as bone marrow. Little is known about these aspects of fibroblasts in cancer, or how they contribute to response or resistance to cancer therapy and metastasis. This project will use genetically engineered mice developed to study collagen-producing fibroblasts in 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 knowledge gained from this project will improve our understanding of what fibroblasts in cancer do, where they come from, and determine if they may be a target for therapy. The findings will be used to guide clinical studies in the future in human disease.	
What species and approximate numbers of animals do you expect to use over what period of time?	This project will use mice. Most mice will be on a commonly used, hardy strain. Approximately 400 will be generated through breeding per year and 300 - 350 of these will be used for procedures or further breeding. Experiments will typically use 8 – 10 animals per group. Pilot studies will use fewer.	

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?	Tumours will be grown in mice that carry genetic modifications allowing the levels, function and fate of fibroblasts to be studied by injecting cancer cells at different sites. Mice will also receive treatments for cancer similar to those already in use in the clinic or of new treatments to see how fibroblasts influence them. The majority of individual procedures conducted in this project are of mild severity with most tumours established using minimally invasive techniques (e.g. direct injection rather than through surgery). Animals experiencing more than mild to moderate levels of severity at any time are expected to be rare. At the end of this project, mice used in this project for tumour models will be killed. New genetically engineered mice made will be cryopreserved and be made available to the wider scientific community.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Fibroblast biology is highly dependent on the local tissue or tumour environment context. It is not possible to accurately model this *in vitro*. Cancer is a systemic disease, influencing multiple organs including sites distant from the tumour. Multiple cell types are recruited, sometimes from distant sites such as bone marrow in cancer. *In vitro* models cannot replicate the systemic effects of cancer. There is evidence fibroblasts participate in these processes in cancer.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Although *in vitro* methods cannot allow the study of fibroblasts in cancer accurately, they can be very useful for preliminary studies to determine what should be studied in mice, significantly reducing the number needed. Careful planning and experimental design coupled to the use of small pilot experiments and will also reduce the numbers required and only the minimum number of mice is needed. Material from experiments will be kept allowing further analysis at a later date, decreasing the need for unneeded repeats of experiments that will use more mice.
3. Refinement Explain the choice of species	As described above, mice are the most refined and least sentient animals enabling the objectives of the

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.	project to be met and the mouse cancer models we plan to use are well established. The genetic modifications made to the mice we will use either have no effect on their biology, or can be tightly regulated to come into effect only when tumours are growing. Where possible, we will follow tumour growth, formation of metastases or fibroblast levels in living animals through detecting levels of light produced by the cancer cells or fibroblasts expressing gene from the firefly. This is very sensitive and often changes in cell numbers can be measured before mice show clinical signs of distress, reducing both harm and numbers of animals used.

Project 10	**Experimental cancer chemotherapy**	
Key Words (max. 5 words)	Xenograft chemotherapy non-genotoxic	
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)	Despite improvements in prevention, diagnosis and treatment, cancer is still the cause of I in 4 deaths in the UK, with 1 in 3 people developing a tumour at some point in their life. New treatments are therefore required urgently. In particular, and unlike most current treatments for cancer, these should not be genotoxic (i.e. they should not induce mutations in the genome that might themselves cause serious health problems during treatment or later in life). Our objective is to demonstrate the efficacies of potential new treatments in pre-clinical models of cancer, such that successful ones can be chosen for trials 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)?	The understanding of tumour initiation and growth, and the responses to novel therapies will help the development of treatments for cancer in humans.	
What species and approximate numbers of animals do you expect to use over what period of time?	Mice will be used for this project. We do not expect to use more than 500 mice over 2 years.	
In the context of what you	We use a standard model of human cancer, in which	

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?	human tumour cells are grown underneath the skin of mice in which the immune system has been manipulated in order for them to accept these so-called xenografts. Tumours growing in this location can readily be measured with callipers, thus allowing regular assessment of the effects of the therapies. Experiments are always terminated before tumours reach a size at which they might cause any adverse effects on the animals' welfare. Because the therapies we are interested in are so novel, we first measure their safety in animals. To do this, we use a very small group size (maybe only two mice) of animals without tumours and start with a very low dose. If we see no welfare problems over the expected duration of the treatment, then we repeat this experiment, using a slightly higher dose, and so on. The dose below the one at which any deviation from normal welfare is observed is defined as the 'maximally tolerated dose' (MTD). For testing the efficacy of the agent in tumour-bearing animals, doses at or below the MTD are used. Animals are killed humanely at the end of the experiment, so that the effects of the therapies can be investigated at the molecular level.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We make extensive use of in vitro systems such as cell and tissue culture in establishing the likely safety and efficacy of novel agents. However, to be effective as a treatment, these agents must work within the whole organism and it is therefore essential to demonstrate this in the intact animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use standard tumour cell lines, whose quality is carefully monitored, in order to achieve as high a graft 'take rate' as we can, and thus avoid wasting any animals. Safety testing is done in very small groups of mice, typically one or two per group. When analysing the tissue distribution and biological lifetime of novel agents, groups of about five mice are used, By taking very small blood samples from the same animal over time, we can reduce the total numbers required but also reduce the variability in the data we collect. For efficacy studies, where we are monitoring tumour growth after treatment, a group size of 10 animals has been found to be robust.
3. Refinement	The animals we use have altered immune systems, but we keep them in individually-ventilated cages

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.	(IVCs) in order to prevent any risk of infection. Tumours are not allowed to grow to a size at which they would impinge on animal welfare. Anaesthesia is used in any procedure there is a possibility of pain (for example, the implantation of an hormone pellet to permit the growth of certain tumour cell types).

<table>
<tr><td>Project 11</td><td>Arginine methylation and tumourigenesis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cancer, genetically engineered mice</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></td><td>No</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>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Cancer is still a major cause of morbidity and mortality in the UK with more than 150,000 deaths a year. Therapeutic regimens have remained unchanged for decades, often involving the targeting of the hyper-proliferative cancer cell through inhibiting DNA and RNA replication. However this approach has many unwanted side effects as normal dividing cells are also targeted. Hence, there is still a continuing need to understand cancer at a molecular level.

Our laboratory is interested in a family of enzymes that have been highly implicated in cancer development and growth using cell culture based approaches. This is important, as these proteins could potentially be targeted for small molecule design in the development of novel anti-cancer therapies. We now want to understand the significance of these enzymes for cancer development, growth and metastasis in a living animal. To do this we will address the following objective:

1) We will determine the effect of altering enzyme expression on tumour development in a living</td></tr>
</table>

	animal, and the biological mechanism by which this occurs. 2) We will understand if and how these enzymes contribute to normal and cancer stem cells (CSCs) function. 3) We will determine how these enzymes contribute to metastatic disease. 4) We will establish if altering the expression of these enzymes synergise with known chemotherapeutic agents in reducing tumour growth in a living 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)?	The primary potential benefits relates to new knowledge about the initiation and progression of tumourigenesis. This will be highly beneficial to both the academic and pre-clinical communities. To achieve this is a timely manner, we aim to disseminate our findings by publishing in academic journals and will do so according to the ARRIVE guidelines. The enzymes we are studying have been recognised as potential drug targets. The knowledge gained in this project will therefore justify the further development of small molecule compounds that specifically inhibit their activity. In the long term, this could greatly benefit the lives of cancer patients.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use approximately 10,000 mice over 5 years. Most of these will be in our breeding programme.
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 experimental protocols have been prospectively assessed as being of moderate severity. These include: 1) Injection of substances that promote gene deletion, enable imaging and measurement of proliferation, or chemotherapeutic agents 2) Blood sampling 3) General anaesthesia for imaging and surgical procedures. 4) Administration of hormones through the surgical insertion of a small pellet or a slow release device. 5) Engraftment of human and murine cancer cell lines 6) Surgical removal of the mammary fat pad under general anaesthesia *Some of the genetically altered mice we will breed*

	will spontaneously generate solid malignancies and/or leukaemia's/lymphomas. However, as these are well-established strains, we will know from previous published studies the expected age of tumour development. To minimise adverse effects, animals will be closely monitored for signs of distress and killed immediately if this occurs. We will end experiments at the first possible humane endpoint that enables the object of the experiments to be achieved with the least possible suffering. We will use suitable anaesthesia and analgesia under veterinary guidance. When required, we will conduct small pilot experiments to obtain information that will minimise the suffering of larger cohorts of animals. After the animal has been killed, we will dissect all relevant tissues and perform extensive biological analysis. Tissue that has not been immediately analysed will be archived for future use by others and us.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Genetic analysis of gene function has been essential for the development of novel therapies for disease, and generally involves the genetic modification of mice. The development of new technologies is now enabling gene editing of human cancer cells in vitro. However, normal development and the pathogenesis of cancer is incredibly complex involving the integration and interplay between numerous cell types. Very few, if any, in vitro cell culture based models are able to recapitulate this interactive environment. Hence, studies using murine models of cancer have been fundamental in enabling a better understanding of the processes that lead to cancer that could not have been achieved otherwise. As a direct consequence of this, new drug targets and insights into the molecular mechanism of cancer establishment and progression have come to light.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In all experiments, appropriate animal cohort size that will enable the generation of statically significant results will be calculated through consultation with a statistician. When possible, experiments will involve a factorial design that will maximise the information obtained from a minimal number of animals. For example, non-invasive

	imaging will enable multiple measurements on the same animal over a period of time. All strains not immediately required for scientific study will be cryopreserved as embryos.
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 are using mice to enable a genetic approach to understand how cancer develops and thus identify novel targets for therapeutic intervention. Mice are the species of choice because there are a large number of widely available genetically modified strains that means that the function of most genes of interest can be studied. In most cases we use a system whereby we can study our gene of choice within a specific tissue through a process called "conditional genetic alteration". By doing so, we minimise the effect of genetic alteration on the animal. A second reason we have chosen to use mice is that the cancer models we will be using have been extensively characterised previously, and develop tumours with similar etiology and molecular profile as that observed in human disease. Moreover, tumour growth rate is predictable, thus fewer mice are required to be used for each experiment. Some of our studies will involve the generation of novel genetically modified animals, however during this process, all animals will be closely monitored for signs of distress and handled in the appropriate manner. In experiments where we transplant cancer cell lines into recipient mice to determine metastasis formation and tumour growth after gene alteration or drug treatment, we will first manipulate cells to express a protein that permits non-invasive imaging. This will enable continuous monitoring of tumour progression in living animals, and hence reduces the number of animals we need to use. Moreover, this design offers the advantage of determining significant differences between tumourigenic growth potential of cell lines before the tumour volume exceeds the limiting size.

Project 12	**Epigenetics of Cell Senescence, Cancer and Ageing**	
Key Words (max. 5 words)	Epigenetics/Cancer/Ageing/Mouse models	
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
		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)	Chromatin is a composite of DNA, protein and other biological molecules that compacts, packages and organizes the cell's DNA into the cell nucleus. Chromatin organization has a profound impact on cell and tissue function. For example, the difference between a liver cell and a muscle cell is determined entirely at the chromatin level. Likewise, changes to chromatin contribute to cancer and age-associated changes to cell and tissue function, including diseases of ageing. The purpose of this application is to use animal models to address the role of chromatin in aspects of aging and cancer biology that cannot otherwise be addressed in cell culture or alternative models. Specifically, we will investigate the role of chromatin in 1) suppression of cancer; 2) tissue ageing and 3) wound healing (impaired wound healing is a common hallmark of tissue ageing).	
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 determine which chromatin regulators are critical determinants of cancer and tissue aging. This will determine whether these regulators represent good targets for biomedical intervention. The models established will provide in vivo models to better understand the function and mechanisms of these chromatin regulators.	

What species and approximate numbers of animals do you expect to use over what period of time?	All studies will use mice. We expect to use approximately 30,000 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?	Animals will be bred to show predisposition to specific cancers, such as melanoma or pancreatic cancer, or will receive a transplant of tumour tissue or cells from mouse or human cancer. Approximately 80% of the mice will not show any adverse effects related to the breeding and not undergo any procedures except for ear notching for identification and genetic testing. These will be kept in normal housing and humanely killed when they are no longer needed for breeding. We will often be able to use tissue samples from these mice after they are killed as normal controls. Some proportion of the animals (approximately 20%) will be predisposed to cancer and will be monitored carefully for clinical symptoms, weight loss, swelling of the abdomen and development of visible or palpable tumours. Mice with tumours will be monitored carefully by trained staff and if the tumours reach 1.2 cm, or become ulcerated or interfere with normal behaviour, mice will be humanely killed and the tissues will be analysed. 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 up to 15% will be humanely killed. At the end of the study, all animals will be euthanised. Some proportion of the animals (approximately 20%) will be predisposed to accelerated ageing or treated with chemicals to promote accelerated ageing in specific tissues. Specific ageing phenotypes include premature hair greying, pancreas, liver or intestinal degeneration, weight loss, hair loss and cataracts. Mice with, suspected of or predisposed to such phenotypes will be closely monitored. Any animals that display signs of general malaise, such as reluctance to eat or move, weight loss up to 15% will be humanely killed. At the end of the study, all animals will be euthanized. Some of the mice (<10%) will be subjected to minor

	skin wounding to assess the wound healing process. We expect to see variations in the rate and efficiency of wound healing, but since wound healing is a normal physiological process and the wounds are minor there should be no debilitating effects. In the event that wounds fail to heal or become infected, leading to discomfort to the mouse, these mice will be euthanised.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Most of our work is done with cell cultures derived from humans or from tissues taken from animals that have been humanely killed, to minimise the amount of work done with live animals. However, some experiments must necessarily be performed in an animal, as cancer cells encounter various organs and tissues, and most often kill via their debilitating effects on tissue function. Mouse represents the best model for human cancer available to us, due to the ability to manipulate the DNA and test the effects of loss or alteration of specific genes on cancer progression.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We perform pilot experiments using only a few animals for new studies, before scaling up to the appropriate numbers for a full study. Numbers are calculated based on the experience of other local research groups 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.
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 genetic models of cancer are widely accepted to be the most closely representative of human cancers. The tumour forms in the correct tissue and spreads via the normal routes and the tumours often progress through the same stages of pre-cancer as in humans. We use state-of-the art genetic models to ensure that the cancer develops in the correct organ/tissues and there are as few side effects as possible due to breeding or treatments. This is done with inducible DNA recombination enzymes that are

	specific to the target tissues of interest and is achieved by breeding these into the genome. Animals will receive anaesthetic and/or analgesic treatments where appropriate. All animals will be monitored regularly for signs of normal behaviour and will be humanely killed if they exhibit moderate adverse signs.

Project 13	**Analysis of stressors nutrition and ageing in cancer**	
Key Words (max. 5 words)	Stress, cancer, treatment, ageing, diagnostics	
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
		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 study psychological stress related signalling mechanisms in healthy ageing and specific aggressive cancers; breast; ovarian and skin cancers to better understand prognosis and improve treatment strategies.	
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 allow us to develop a better understanding of the signalling mechanisms involved in stress, depression, cancer and ageing and further help us understand the role of the gut bacteria in treatment efficacy. The data produced will provide pre-clinical evidence of the mechanism of action and efficacy of specific interventions in small animal models of cancer. Furthermore, the influence of psychological stress and the gut bacteria are both recognised as important components of other human pathologies such as other cancers and the information obtained will be of benefit to medicine in general. Ultimately, the results of this research will allow the improved cancer diagnosis and response to drug treatments.	
What species and approximate numbers of animals do you expect to use	We expect to use mice and approximately 1400 over the 5 year period of this license.	

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?	Administration of cancer cells into the mammary fat pad or subcutaneously No significant risk of adverse effects. Each animal will carry a single tumour, the mean diameter will not exceed 1.2 cm in mice or 1.5 for therapeutic studies (Workman et aI,, 2010). (2) Administration of Investigative Agent(s) No significant risk of adverse effects. The P1 has significant experience with drug and hormone administration into mice. If an adverse event should occur (highly unlikely—but if an IP injection accidently hits an organ) the animal will be culled immediately. (3) Assessment and application of stress This method is considered to be largely psychological in nature resulting in elevations in corticosterone. Because stress is an adverse effect then we will monitor weight loss and signs of distress e.g. ruffled fur ungroomed appearance. If weight loss decreases below 20% the animal will be culled immediately. All animals will be culled by schedule I killing The severity levels are mild.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We will perform analyse sin cell culture models prior to any animal research. The cell culture models will allow us to determine doses/concentrations and effects and which of the effects can be safely administered to mice. Investigations using these models will allow some cellular mechanisms of cancer to be studied in detail as well as the effectiveness of novel diagnostics and therapies prior to their use in mouse models, thereby allowing us to reduce the numbers of animals used. However, animal models of cancer must ultimately be used as these are the models which most closely resemble the diseases observed in patients. More specifically, these models are able to reproduce the interactions between different physiological systems which occur in cancer such as the interactions between the immune and nervous systems
2. Reduction Explain how you will assure the use of minimum numbers	We are reducing the number of animals by performing cell culture experiments before any animal work is carried out. Power calculations have been performed to ensure that we use the minimum number of animals required to generate meaningful and reliable

of animals	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.	For cancer research, the current models available are 1) xenograft (human cells in mouse models, 2) syngeneic models e.g. taking mouse mammary tumours and placing them in another mouse and 3) genetically modified mouse models (e.g. adding or removing genes). All of these models have been validated and their use widely reported in the literature. We will use xenografts because we can directly look at the effects of stress on cancer cells in the absence of an immune system. Syngeneic and transgenic models are required for us to investigate our questions in the presence of an intact immune system we have carefully considered each of the procedures described in this application in order to minimise pain and distress experienced by animals, and to enhance their well-being. Our animals are housed in the best possible conditions and handled by competent and experienced staff. For example, we provide enrichment such as reformed cardboard housing in our mouse cages. Within the research laboratory, the staff are experienced and well trained and new staff and students are carefully supervised until they are confident and competent in the handling of animals and in in vivo procedures. In line with Workman et al (2010) paper 'Guidelines for the welfare and use of animals in cancer research' British Journal of Cancer (2010) 102, 1555—1 577 we will inject the minimum number of cells in the smallest volume e.g. 1—5 million cells in 100 p1 into the mammary fatpad. Assessment of the size of superficial tumours using callipers (usually of two diameters at right angles) will be used. Measurement variations will be minimised by ensuring that the same well-trained technician is involved for the duration of the study. Removing and weighing tumours at the end of a study will provide an additional objective endpoint, which avoids errors due to variations in tumour shapes and estimations of volume. For an animal carrying a single tumour, the mean diameter will not exceed 1.2 cm in mice, or 1.5 cm for therapeutic studies. We will also look for signs of distress including: ruffled fur, ungroomed appearance, less social interactions with other mice, increase heart rate and inability to walk and move. Any mice showing signs of distress will be immediately euthanised.

Project 14	**Tumour progression in Zebrafish models of cancer**	
Key Words (max. 5 words)	Cancer, Cell Signalling, Biosensor, Tumour Microenvironment, Optical Projection Tomography (OPT)	
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)	Cancers figure among the leading causes of death worldwide, accounting for 8.2 million deaths in 2012. It is expected that annual cancer cases will rise from 14 million in 2012 to 22 within the next two decades. However, the mechanisms that cause, or protect against, cancer are insufficiently defined, and there is a continued need for new therapeutic approaches. We will elucidate the spatial and temporal signalling molecules and mechanisms important for cancer initiation and progression. We will also develop and optimise a novel three dimensional global imaging platform for studying cancer in live zebrafish. This work will help to identify new therapies or therapeutic targets for 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)?	This project will provide new insights into basic biology and cancer progression. The biological methodologies integral to this project could benefit a wide range of life scientists wishing to study live juvenile/adult zebrafish models that exhibit an adaptive immune system and enable studies of biological processes and more realistic disease	

	progression over timescales longer than the few day limit for zebrafish embryos. The proposed imaging of cancer processes in vivo, particularly tumour-vasculature interactions, tumour progression and metastasis would benefit both cancer biologists and oncologists, who could exploit this capability for drug development.
What species and approximate numbers of animals do you expect to use over what period of time?	This project requires transparent organisms. Zebrafish can be bred to be transparent and are vertebrates – and so represent better human biology than other transparent organisms. In addition, zebrafish are highly amenable towards genetic manipulation. Zebrafish also allow studies in simpler vertebrate organisms. We expect to use 22,500 zebrafsh over the duration of this project as described below: Breeding - 10,000 Generation of genetically modified zebrafish - 10,000 Juvenile/Adult zebrafish used in genetic models for studying cancer progression - 2,500 Genetically modified Zebrafish have an inherently lower viability rate compared to mammals (such as mice and rats) (~75%). The viability of embryos undergoing genetic modification procedures can be (~1 – 20%) depending on the gene(s) being manipulated. Death usually occurs before 5 days post fertilisation (dpf), however it can be longer due to embryonic development. Regulations require accounting for all embryos >5 dpf. Furthermore, regulations require that all adult zebrafish are culled at 2 years post fertilisation.
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 zebrafish will not undergo procedures that will inflict harm. Instead these zebrafish will simply be bred and may carry a mutated gene; some fish will be induced to develop a tumour, and some fish will be exposed to anti-cancer therapeutics. Fish will then be monitored using non-invasive imaging techniques for the development of tumours. Based on our experience, adverse effects are anticipated to be very limited in all our protocols and where they do occur to be very brief in duration. Adverse effects that may occur include difficulty breathing, abnormal colouration, abnormal swimming, feeding or schooling behaviour. All our protocols have a severity level of mild or moderate. All animals will be humanely killed at the end, and/or when signs of

	discomfort or pain are manifested.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	While much has been learned from cells in culture, it is increasingly accepted that the development of therapies will benefit from *in vivo* studies, since biological processes can differ significantly between *in vitro* and *in vivo* contexts, and from whole body imaging since global responses can determine the ultimate phenotype. This is particularly true for cancer. For cancer the tumour/host interface is critical for tumour development and metastasis and is an important target for drug discovery. In general, studies in live disease models are increasingly important for drug discovery, reducing time to failure for unsuccessful compounds and providing opportunities to discover off-target effects.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In all cases the minimum number of animals will be used in order to obtain statistically significant results, based upon power calculations and the previous literature based on similar animal models. Where appropriate, pilot studies will be carried out (n=3) in order to determine the correct experimental conditions, in order , for example, to ensure adequate levels of a florescent signal necessary for readout of a particular biosensor or imaging of the tumour microenvironment.
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 visualise internal processes requires transparent organisms. Zebrafish are particularly useful because they can be bred to be transparent and are vertebrates – and so represent better human biology than other transparent organisms. They are increasingly used for biomedical research and many zebrafish disease models have been produced in the adult, including for cancer. Unfortunately the use of embryos precludes full studies of disease progression such as tumour development because of multiple factors including immature vasculature and immune systems. The models we propose to use are well-established, the procedures involved have been refined and are known to be well-tolerated and cause minimal pain or discomfort. All animal studies will be performed by highly experienced and skilled animal investigators, and the advice of veterinary surgeons will be sought when applicable. Measures will be taken at each step of the work to

	prevent pain, discomfort or other adverse effects, and to treat any such signs should they arise. Experiments will only be of sufficient duration to achieve the objectives of the work and at all times, persistence of adverse effects and any suffering in animals will be avoided by immediately withdrawing such animals from the study and killing them humanely.

Project 15	**Investigating Human Pancreatic Cancer in Mice**	
Key Words (max. 5 words)	Pancreatic cancer, genes, therapy	
Expected duration of the project (yrs)	Five	
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 seek to understand the molecular basis for pancreatic cancer development and spread in the body. We study the genes that go wrong in pancreatic cancer and we apply this knowledge to understand how cancers develop and spread to other sites in the body, and how we might best treat 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)?	We hope to uncover and test new targets for development of medicinal therapies against the spread of cancer. Our project concentrates on pancreatic cancer, but our work could apply to many cancer types.	
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use up to 6,000 mice per year over 5 years for this project. Around 80% of these will not undergo any 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,	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. Approximately 80% of the mice will not show any adverse effects related to the breeding and not undergo any procedures except for ear notching for identification and genetic testing. These will be kept in normal housing and humanely killed when they are no longer needed for breeding. We will often be able to use tissue samples from these mice after they are killed as normal controls. Some proportion of the animals (approximately 20%) 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, get bigger, or have greater consequence than, accepted guideline limits for cancer studies 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 euthanized.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Pancreatic cancer is a very complex cancer that involves a number of different cell types e.g. cancer cells, immune cells, blood vessels. In addition, pancreatic tumour cells grow within a particularly dense matrix that plays a major role in tumour development, growth and spread, and stops drugs from working properly. Current non-animal models cannot reproduce this situation and are not appropriate for studies to understand pancreatic cancer progression or for testing of new drugs.
2. Reduction	We perform pilot experiments using only a few

Explain how you will assure the use of minimum numbers of animals	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.
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 genetic models of cancer are widely accepted to be the most closely representative of human cancers. The tumour forms in the correct tissue and spreads via the normal routes and the tumours often progress through the same stages of pre-cancer as in humans. We use state-of-the art genetic models to ensure that the cancer develops in the correct organ/tissues and there are as few side effects as possible due to breeding or treatments. This is done using mice that are engineered to produce enzymes, specifically in the target issue of interest, which can cut out or activate genes. Animals will receive anaesthetic and/or analgesic treatments where appropriate. All animals will be monitored regularly for signs of normal behaviour and will be humanely killed if they exhibit moderate adverse signs.

Project 16	**Analysing cancer metastasis and therapy failure**	
Key Words (max. 5 words)	Cancer, Cell Movement, Imaging, Metastasis, Treatment	
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 is a devastating disease that either directly or indirectly affects almost everyone in the UK. The most deadly aspects of the disease are when it spreads and when it stops responding to the therapies that are available. This project aims to understand these processes in more detail. In particular, we want to understand how cancer cells move through the different tissues in the body. Further, we want to identify ways of stopping this process. We already know that cancer cells can co-opt non-cancerous cells to assist their spread through the body. We would like to learn more about this so that we might stop the 'communication' between cancer cells and normal cells. This could also reduce the spread of cancer. Finally, many existing therapies work for a while but then fail. We would like to understand this process. We think it is linked to the ability of cancer cells to 'communicate' with non-cancerous cells. This links this part of the project to our efforts to understand how cancers 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	The potential benefit of this project is the improved treatment of cancer patients. We hope information generated in this work will inform future clinical trials and lead to improved outcomes for people with	

project)?	cancer. In addition, this work will enhance our understanding of how the mammalian body works and may shed light on other diseases, such as fibrosis.
What species and approximate numbers of animals do you expect to use over what period of time?	We anticipate using 6000 – 9000 mice over a 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?	Many of the mice will be used for tumour studies or for comparison with mice with cancer (scientific controls). Some mice may be genetically altered to make them more cancer prone (similar to some cancer prone families) or to help work out whether certain genes would be good targets for anti-cancer drugs. Clearly, the development of tumours is an adverse effect, but this is necessary to study cancer. The treatment of mice with cancer therapies and/or surgery could also lead to adverse effects similar to the side-effects experienced by cancer patients; once again this is necessary to improve our understanding of why some cancer treatments fail and how they can be improved. Most of the animals used will experience sub-threshold or mild severity procedures; we expect 20-30% to experience moderate severity procedures, such as larger tumours or surgical procedures to remove tumours. Imaging will be performed under anaesthesia with animals monitored closely while recovering from the anaesthetic, we expect minimal adverse effects from these procedures. Once we have obtained the information we need from the experiment the mice will be humanely euthanized.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our group makes extensive use of replacements; including 'organotypic' models, computational models, analysis of patient material, and conventional cell biology. This means that we have already explored our hypotheses in great detail before we consider doing using mice. However, we cannot completely mimic the complex tissues through which cancers spread using these techniques. Therefore it is sometimes necessary to perform experiments with mice. In addition, the part of our work that aim to study how cancer cells communicate with non-cancerous cells include analysis of the immune-system and exploration of whether the immune-

	system can be targeted to cancer cells. This is an area of great therapeutic potential with new drugs licenced in the last year, however it cannot be replicated in any culture systems and this means that mouse experiments are required.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The use of numerous replacement systems for many of our experiments means that we have well formulated hypotheses before we begin mouse studies. This means we have realistic ideas about what to expect and can perform statistical 'power' calculations to work out how many mice are required to give a clear answer to the question we are asking. These calculations are assisted by a PhD qualified statistician. We also try to maximise the information we get from each mouse. For example, by using imaging to measure tumour size in the same animal regularly we get a much clear picture of how a tumour responds to a therapy than by comparing the tumour size of mice treated with or without drug on a single day. Analysis of blood and other tissues can further increase the amount of useful information.
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 how cancer cells move through our bodies it is important to use animals with fairly similar bodies, such as mammals. Much of our work involves understanding breast cancer. This means that our studies require animals with mammary glands, and by definition these are mammals. Mice are the simplest mammal that are practical to use (this judgement is based on husbandry, breeding rates, and an extensive existing knowledge base). Our group is actively engaged in trying to refine procedures to minimise adverse effects. We were the first group in the UK to implement a new less bulky imaging device. We liaise closely with vets to include the most suitable analgesia in our experiments. In addition, we routinely seek to use environmental stimulation (such as little houses) in mouse husbandry.

Project 17	**Elucidating Novel Actionable Mutations in Cancer**	
Key Words (max. 5 words)	Enzymes, Genetics, Cancer Drivers, Drug Pathways, Tumour-Suppressing Enzymes	
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)	The objective of the project is to identify and verify the importance of 3 novel enzymes that are aberrantly activated in cancer cells but not normal cells, and assess if inhibiting these enzymes results in specific killing of the cancer cells, but not the normal cells. Other novel enzymes may be tested after extensive in vitro work.	
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)?	If these results are positive they could lead to treating certain patients that have increased expression or mutations in these enzymes with already approved precision medicines, leading to significant decreases in tumours for the patients and increased life-span without toxic side effects of standard chemotherapies.	
What species and approximate numbers of animals do you expect to use over what period of time?	Mice will be used; approximately 1800 over the course 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	Adverse effects due to tumour growth will be minimal and tumours will not be allowed to grow beyond 1.2 cm^3. Any animals which show marked suffering will be culled. The dose and duration of anticancer drug administration should result in only subtle non-	

end?	specific signs indicative of loss of condition, such as mild reduction in activity. Animals will be closely monitored and killed if they show signs of ill health. All animals will be killed 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	The mouse represents the lowest level of sentience that is a strong predictor for clinical outcomes in patients. Clinical trials targeting novel enzymes cannot be carried out in humans without prior testing and positive results in the proposed models. Whenever possible mathematical models and in silico/in vitro approaches will be used to verify all data and hypotheses prior to performing studies in mice.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In order to use the minimum number of animals, pilot experiments will be conducted in the first instance, followed by statistical analysis to make sure the minimum numbers of animals are used that still allow for statistically meaningful data to be produced. The expertise of specialised biostatisticians is available within the institute and will aid in experimental design.
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 tumourigenesis have correlated well with responses in human to cancer therapies and reduction in tumour growth, and much more accurately predict responses to therapies than in vitro studies or use of other species to model tumour responses. Furthermore, minimal invasive procedures will be used to minimise risk to the animal, for example by monitoring tumour sizes by calliper measurement, and improved instrumentation and improved control of pain used whenever animals are distressed.

Project 18	**Aspects of cancer regulation**	
Key Words (max. 5 words)	Cancer, Macrophage, dormancy, lncRNAs	
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)	Our aim is to understand what drives the progression of cancers and how host responses are involved. We are interested in both the host immune cells, specifically macrophage cells, and how they turn against the host and work for the tumour, and also how the tumour environment maintains a cancer cell in a state of dormancy or flips the switch to allow proliferation, ultimately leading to fatal metastases. We are also interested in how non-coding RNAs are involved in cancer 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)?	We anticipate finding a selection of genes that will further our understanding of the mechanisms involved with cancer immunology and cancer dormancy. We also hope to identify cancer related functions of non-coding RNAs. Some of these findings could ultimately lead to novel therapies for cancer treatment and/or preventions. In addition we will publish our findings in peer reviewed journals, thereby sharing with the scientific community so that our data and methods can help others working on similar projects. Our genetically modified mice will also be valuable to the wider community, reducing the need for others to expand animals recreating them.	

What species and approximate numbers of animals do you expect to use over what period of time?	Mice Approx. 4000 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?	Due to the nature of the projects the mice will undergo surgical procedures and/or develop tumours/malignancies, however these are anticipated to cause only mild discomfort, and pain relief will be given where necessary. Their progress will be closely monitored to ensure they are not suffering beyond expected. All animals will be humanly killed at the end of the experiment, or transgenic mice will be kept alive in the authorised establishment or transferred to alternative authorised protocols.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The understanding of cancer development, including host interactions with cancer cells is a necessary project if we are to translate our findings to a potential therapy. The early stages of cancer, metastasis, the immune response and cell to cell interactions are impossible to study in vitro, nor in other models such as insects and fish
2. Reduction Explain how you will assure the use of minimum numbers of animals	The understanding of cancer development, including host interactions with cancer cells is a necessary project if we are to translate our findings to a potential therapy. The early stages of cancer, metastasis, the immune response and cell to cell interactions are impossible to study in vitro, nor in other models such as insects and fish
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 have chosen to do this work in mice. These are best suited for this project as they capture human disease relatively faithfully. In addition there are a large number of models available to us, and there is compatibility with the commonly used cell-lines of mouse breast tumour. We will minimise the animal suffering by monitoring the growth of the tumour and ensure it does not extend beyond the recommended guidelines. The surgeries will be performed under published best practise guidelines, or where we have modified these to reduce suffering further.

Project 19	**Cellular and molecular haematopoietic dynamics in health and disease**	
Key Words (max. 5 words)	Stem cells, haematopoiesis, transplantation, intravital microscopy, stem cell niche	
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
		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)	Most blood cells are short lived and are constantly replaced by the balanced production of new progeny arising from blood stem cells. We aim to understand the mechanisms regulating such balance in order to guide the development of new therapies for blood disorders such as leukaemia or bone marrow failure. Still very little is known about the molecular mechanisms regulating blood stem cell function and especially about the signals integrated by the stem cells' microenvironment. As a result, it is still impossible to efficiently culture and expand blood stem cells in vitro. We have developed a new methodology based on an advanced type of in vivo imaging technique called two-photon microscopy. This is the least invasive technique allowing tracking fluorescently labelled blood stem cells interaction with bone marrow components in real time and at subcellular resolution in the mouse skull. We will use this technique to understand the mechanisms regulating blood stem cell fate and in particular the relationship between cell positioning and function.	

	We will investigate what factors lead stem cell daughters to remain in the niche, expanding the stem cell pool, or to change location and give rise to mature blood cells. We will observe how incorrect cell localization results in onset of 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)?	Blood stem cells are widely used for bone marrow transplantation, however their supply is limited and to date no one has successfully expanded blood stem cells in vitro. Our work will lead to improved culture techniques and benefit a higher number of patients awaiting bone marrow transplantation. It has recently been proposed that leukaemia cells compete with blood stem cells for niche space, a hypothesis that can be tested uniquely through in vivo studies. Our results will lead to the development of new leukaemia treatments aiming at eradicating the disease while preserving, and ideally strengthening, normal blood stem cells. Finally, understanding blood stem cells activity and requirements will moreover allow developing preventative approaches to enable healthy ageing, which will benefit the whole population.
What species and approximate numbers of animals do you expect to use over what period of time?	In vivo studies are the best approach to shed light on such mechanisms and the laboratory mouse is the model of choice because of its size and relative similarity to human physiology and because it is amenable to controlled genetic modifications. Analysis of multiple animals will be required in order to obtain statistically significant results. Three to four recipient mice need to be analyzed with in vivo imaging in order to draw conclusions on specific localization and proliferation of the injected cells. Functional fitness of blood precursor cells needs to be analyzed in groups of five-ten recipient mice due to mouse-to-mouse variation, present even within isogenic strains. Moreover, stem cells are rare and require working with a considerable number of donor animals when highly purified stem cell populations are analysed. For example, stem cells from four donors are typically sufficient to be injected and imaged in one recipient mouse. We expect we will be using approximately 8000 animals over the 5 years of the study, including both purchased wild type mice and genetically modified reporter lines bred in house that allow visualisation of haematopoietic and stroma

	cells. Of these mice, approximately 3000 will undergo cutting edge intravital microscopy techniques, which will allow addressing questions on the biology of HSCs that are critical for the development of therapeutic and preventive strategies and could not be otherwise answered.
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 levels of severity range from mild to moderate as all the techniques used in the project are well established and keep being refined. The expected adverse effects derive from preparative chemotherapy in the case that the transplanted haematopoietic cells do not engraft appropriately, from unexpected accelerated development of leukaemia, and from complications arising from surgery to allow intravital microscopy. The details of these adverse effects are described in each protocol. In summary, all mice will be monitored carefully, aseptic surgical techniques will be used, appropriate analgesics and anaesthetics will be administered and humane end points will be enforced if the severity levels experienced exceed those expected.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In the same way that a comfortable environment improves workers' performance, correct positioning of blood stem cells in specific niches within the bone marrow space is crucial to ensure their correct functioning. Still very little is known about the molecular mechanisms regulating blood stem cell function and especially about the signals integrated by the stem cells' microenvironment. As a result, it is still impossible to efficiently culture and expand blood stem cells in vitro. In vivo studies are the best approach to shed light on such mechanisms and the laboratory mouse is the model of choice because of its size and relative similarity to human physiology and because it is amenable to controlled genetic modifications. The principles of HSC biology highlighted by our work will directly inform in vitro HSC culture protocols that we and others are currently testing, and therefore over time in vivo experiments will be progressively replaced with improved experimental models based on in vitro co-culture of HSCs with the appropriate stroma cells and in the presence of the appropriate molecular signals.
2. Reduction	Only the number of animals necessary to generate

Explain how you will assure the use of minimum numbers of animals	statistically robust data will be used. Power calculations will establish how many test and control animals should be studied to observe a certain predicted difference. If during the course of the experiment the actual difference observed is higher than the predicted, a lower number of animals will be necessary to acquire statistically significant data and therefore it will be reduced. Efficient data analysis performed almost simultaneously to acquisition allows us to adjust and, whenever possible, to reduce the number of animals that need to be analysed. Moreover, by monitoring the same mice longitudinally, over multiple imaging sessions, we collect more robust data than we would by imaging each mouse only once, and having to use lager cohorts to compensate for the larger variability. Finally, a small percentage of mice undergoing cell transplantation (protocol E2) may present a phenotype that could only be explained by intravital microscopy. By continuing their use under protocol E4 we will reduce the overall number of mice used by avoiding to re-start an experiment of the same size under protocol E4.
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 smallest vertebrate with a haematopoietic system homolog to the human and it is therefore the most refined animal model that we can use to understand aspects of HSC biology that are impossible to address with non-animal experimental models. We will ensure the welfare of the animals by monitoring them closely and by constantly refining our techniques, for which we already have an excellent track record. For example we have refined our intravital microscopy techniques to allow longitudinal imaging of mice, we have refined our cell transplantation techniques to minimise the occurrence of side effects due to the conditioning or the development of leukaemia, and we will keep collaborating with the local vets and NACWOs to continue further refinement. For example, we have recently developed Standard Operating Procedures and procedures that will ensure the mice are kept in the microscopy room at the same temperature and lighting conditions as in the animal facility in order to minimise their stress.

<table>
<tr><td>Project 20</td><td colspan="3">Imaging of Cell Therapy In Tumour Models</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Tumour, cell therapy, imaging</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 Years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td colspan="3"><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></td><td>No</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 colspan="3">Cancer affects thousands of people in the UK every year and is a major cause of mortality and morbidity. Cellular therapies (adoptive cell transfer or stem cell targeting) have the ability to treat cancers throughout the body but needs more rigorous testing before being applied to humans. The aim of this work is to use novel non-invasive imaging techniques to 1) identify the distribution and life span of transplanted cells for optimal application to specific cancers and identify dosing regimens, 2) use these imaging developments to assess if cell therapies can be utilized as cancer therapies reducing cancer burden in a range of tumour types and stages. By using imaging we can develop translatable methods with better designed trials with the ultimate goal of reducing/eradicating tumour burden, extending remission and survival rates and preventing metastatic spread.</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 colspan="3">The benefits of this work primarily focus on a range of different cancers and the treatment of such. The impact of techniques for the development of tracking cell therapies by imaging has a far wider benefit as cell therapies have the potential to be utilized in curing a vast number of diseases. The translation of the novel imaging methods to assess cancer can also be applied to other diseased tissues so our work will contribute to disease progression in a range of</td></tr>
</table>

	organs.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice and over the five years we will use a maximum of 6000 animals.
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 we are modelling progressive cancer our animals will increasingly demonstrate signs of disease over time. However using imaging we can closely manage these signs and generally can define disease at a much earlier stage and therefore we ensure that the animals do not undergo any undue suffering.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The delivery/migration of cell therapies to different types of cancer in different organs systems and at different stages of disease is complex. It involves many different processes and cell to cell interactions which need to be understood before their use in humans. It is impossible to model such complexity without using animal models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	It is possible to calculate the numbers of animals required for experimentation based on previous data. Imaging lets animals be used as their own control, allowing paired comparisons, and imaging is inherently sequential, using fewer animals to achieve the same statistical power as conventional designs. In all cases we ensure that we have calculated the minimum number of animals required for the experiment to give us useful data. This approach also reduces the likelihood that the animal experiment would have to be repeated.
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.	Using imaging we regularly refine the disease models we use to reduce animal harm and to improve the effectiveness of our models. We can also stage disease and stop experiments before external clinical signs appear, thus limiting disease severity. We also regularly monitor body weight, body condition, food and fluid intake of animals as a measure of disease; we set strict limits to ensure that there is limited harm to the animals used.

Project 21	**Pancreatic cancer – improving our understanding and therapeutic options**	
Key Words (max. 5 words)	Pancreatic Cancer, Therapeutic, Genotype/Phenotype	
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 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 species and approximate numbers of animals do you expect to use 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 level 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 trial, meaning that the effects are known and recognised, and can be treated palliatively. All animals are to be humanely killed at the end of each study, or if they do not respond to therapeutic or palliative care.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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 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 22	**Targeting the mechanisms of tumour vascularisation**
Key Words	cancer, metastasis, tumour stroma, angiogenesis, therapy

- Summarise your project (1-2 sentences)

Tumours have to recruit a blood supply in order to grow. We will address the mechanisms that allow cancer cells to recruit a blood supply and we will address how to target this process effectively in order to kill cancer cells.

- Objectives: Explain why you are doing this project. Describe the scientific unknown(s) or clinical or service need you are addressing. Give a brief scientific background or other explanation of why the work is needed.

We know that cancer cells need to recruit a blood supply in order to grow. Therefore, drugs have been developed that can block blood vessel recruitment in cancer. These drugs have been shown to be effective in patients and are now used to treat several cancer types, including bowel, cervical, liver, ovarian, kidney and stomach cancer. However, there are some significant problems with these drugs.

Firstly, whilst they are effective in some cancer types (including those listed above) they do not work well against other cancer types (including breast cancer, prostate cancer, pancreatic cancer and skin cancer). Secondly, even in cancers where these drugs can be effective, not all of the patients respond to the treatment. Thirdly, even when patients respond to these drugs initially, the benefit is only transient, because the cancers somehow learn how to survive the therapy.

So, although targeting blood vessel recruitment can suppress cancer in patients, the current treatments we have are not as effective as we would like. The work described in this project is needed, because it will address how to target blood vessel recruitment more effectively in cancer patients. In the long term, we expect that this work will improve treatment outcomes in cancer.

- Outline the general project plan.

We will use *in vitro* model systems and the analysis of patient samples to determine genes that are involved in the process of blood vessel recruitment by tumours. We will then test the role of these genes in blood vessel recruitment by performing animal experiments. We will also test whether targeting these genes suppresses tumour growth by performing animal experiments.

- Predicted harms: Give a brief description of the procedures to be applied to the animals used in this project and describe the expected adverse effects.

We will use mouse models of cancer for our studies. Cancer cells will be introduced into mice by direct injection of cells: (a) under the skin, and (b) into the mammary gland. Cancer cells will also be introduced in a way that results in the formation of tumours within internal organs: (a) injection of cells into the tail vein to produce lung tumours, (b) by injection of cells into the liver or spleen to produce

liver tumours, and (c) by injection of cells into the caecum to produce colon tumours. In some cases we may also surgically remove a tumour growing under the skin, in the mammary fat pad or in the colon and then allow the mouse to recover. These mice may then develop spontaneous metastases (where tumour cells start to grow in other parts of the body).

The process of introducing cells into mice is performed in such a way that distress and pain are minimised. Implantation of tumour cells into internal organs, when performed by a competent person and using appropriate anaesthesia and analagesia, can be achieved with minimal distress to the animal.

The growth of a tumour mass in superficial sites such as the skin and mammary fat pad may cause some minor discomfort. Ulceration of the skin at the site of the tumour mass can happen, but is rare (~ 5% of mice).

As long as the tumour burden is low, the growth of tumours within internal organs, including liver, lung and colon is generally well-tolerated in mice. However, complications, such as discomfort, loss of weight, breathlessness (lung only) and bowel obstruction (colon only) can manifest in the mice when the tumour load becomes greater. However, we minimise the risk of these complications by (a) monitoring tumour burden and mouse condition on a regular basis, and (b) using predefined endpoints that are designed to pre-empt and avoid the onset of these complications.

Tumour-bearing and tumour-free mice will also be treated with drugs, but these will be used at doses that are not expected to induce adverse effects. In the case that oestrogen-dependant cancer cells are utilised, mice may also be implanted with oestrogen pellets. These pellets can cause sometimes cause bladder obstruction, but the mice will be closely monitored for signs of this.

- Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.

Cancers require a blood supply in order to grow, but our knowledge of how they achieve this is incomplete. From this project we will (a) gain a greater understanding regarding how tumours obtain a blood supply, and (b) identify new strategies for targeting the tumour's blood supply and suppressing tumour growth. The overarching aim is to find new and more effective treatment strategies for patients.

- Estimate the numbers of animals of each species to be used; explain what types of animal will be used and why you chose the particular types of animal. Explain how you will ensure that you use the minimum number of animals.

We will use mice for these experiments, because mice are a well-established model system for cancer studies. We anticipate using no more than 5,625 mice over the 5 years. We will also employ: (a) *in vitro* experiments and (b) the analysis of samples obtained from patients. Only if these experiments suggest that an *in vivo* experiment is warranted will we then proceed to use animals. Our experiments will be designed so that we always use the minimum numbers of animals

necessary to detect a statistically significant difference between experimental groups.

- Demonstration of compliance with the 3Rs: State why you have to use animals and cannot use non-animal alternatives. Where appropriate, say how you will use non-animal studies in parallel with the project

Much of our research programme employs (a) *in vitro* experiments and (b) the analysis of samples obtained from patients. These studies give us new information about cancer. But, we need to perform animal experiments to confirm the physiological relevance of our findings. This is because there are currently no suitable *in vitro* models that can adequately model the complexities of the *in vivo* tumour environment. However, we only perform animal experiments if data from the other parts of our programme (i.e. *in vitro* experiments or patient samples) tells us that an animal experiment is warranted.

- Explain why the protocols and the way they are carried out should involve the least suffering.

We will employ several strategies in order to reduce the chance of any animal suffering. First, all techniques will be performed in a way that is designed to minimise pain to the animal and we will use anaesthesia and analgesia whenever it is deemed necessary. Second, wherever possible, mice will be culled at pre-defined endpoints that are designed to pre-empt and avoid the onset of any adverse effects. Third, tumour burden will be closely monitored and will not be allowed to exceed the defined limits. Fourth, drugs will be administered at doses below the level expected to induce toxicity. Fifth, animals will be observed daily for any signs of distress. Depending on the severity and recoverability of the condition, any animal found to be suffering will either be treated appropriately or will be humanely killed.

<table>
<tr><td>Project 23</td><td>Defining mechanisms of cancer progression and dissemination</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cancer, Metastasis, Microenvironment, Imaging</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></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>Metastatic dissemination and therapeutic resistance are the most life-threatening aspects of cancer and represent the most significant challenges to manage the disease; however, our knowledge of the mechanisms regulating them is still very limited. A better understanding of these processes will inform the development of new clinical agents and increase our knowledge of the disease.

Solid tumours are not just cancer cells, but are also composed of multiple non-cancerous cell types and a complex extracellular matrix that form what is known as the “tumour microenvironment”. Cells from the tumour microenvironment include immune cells, endothelial cells forming blood and lymphatic vessels, adipocytes and fibroblasts, among others. These cells are generally subverted into performing tumour promoting functions and therefore actively participate in many of the hallmarks of cancer including invasion, angiogenesis, immune evasion, survival and proliferation. Thus, it is being increasingly acknowledged that targeting the tumour microenvironment represents an interesting opportunity for therapeutic intervention. In order to design such approaches, a thorough</td></tr>
</table>

	characterization of the mechanisms governing their emergence, maintenance and tumour promoting abilities is still necessary. The overall aim of this project is to define mechanisms of cancer progression, dissemination and therapeutic resistance. We will study cancer cells and non-cancerous cells (i.e. the tumour microenvironment). In particular, we will investigate relevant questions within this field: • How do cancer cells interact and perturb the tumour microenvironment in primary tumour and metastases? • Can we identify the molecular mechanisms that regulate tumour progression and dissemination? • How does the tumour microenvironment participate in cancer progression, dissemination and therapeutic response? • Can we identify genes involved in these processes (either in cancer cells or in non-cancerous 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)?	The benefits of this project include: 1) A better understanding of cancer progression, dissemination and response to treatment. We expect that our analyses will lead to the identification of molecular targets involved in these processes. This may suggest therapeutic opportunities to develop future anti-cancer agents and improved cancer prognosis that would enable better clinical management of patients. 2) This project may improve the efficiency of translational research and clinical trials. The characterization, optimization and development of mouse models and approaches, and a more detailed understanding of metastasis and the participation of non-cancerous cells in cancer may lead to better pre-clinical models. 3) This project will benefit the basic research community by contributing to our knowledge of mammalian physiology and cell biology. 4) An improved understanding of the details of the contribution of stromal cells to tumoural processes should enable better *in vitro* models to be developed. This may ultimate reduce the number of animals used in research.

What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice that will be generally kept for 12-18 months. We expect to use approximately 7,000 mice over a period of five 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?	We will use the animals to perform cancer studies. Animals are not expected to show an overtly harmful phenotype, other than tumour development. Tumours will be primarily subcutaneous tumours and breast tumours. In certain cases, animals may also develop metastatic tumours in lung, liver and bone. Tumour burden will be limited to the minimum required for a valid scientific outcome. Occasionally, tumours may ulcerate and very rarely they will compromise locomotion. Depending on the tumour model, cancer cells may metastasise to distant organs. Metastasis may present as (e.g.) weight loss, palpable internal tumours or lymph nodes, compromised respiration. Animal suffering will be minimised by making every effort to keep the tumour models employed at the subclinical levels. Other adverse effects associated to the experimental manipulations described in this project include risk of infection and minor pain or discomfort that will be dealt with using aseptic techniques, antibiotics and analgesics. Toxicity may arise from the use of anticancer agents and radiation. This is not expected to be a regular occurrence as they are delivered at previously determined well-tolerated doses. Animals will be humanely killed at the end of each procedure. We have also indicated several guidelines that regulate when animals should be humanely killed before the end of the procedure.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are many aspects of tumours that cannot be adequately modelled *in vitro*. These include the presence of a wide range of stromal cells which can influence tumour growth, dissemination and response to therapeutic agents. Furthermore, metastatic dissemination is a multi-layered and complex process involving different tissues that, at present, can only be fully studied *in vivo*.

2. Reduction Explain how you will assure the use of minimum numbers of animals	We minimize breeding to just mouse lines that are essential for our research and not commercially available. We use statistical power calculations to help determine the most appropriate number of mice to use to test an experimental hypothesis. We implement techniques that allow us to study tumoural processes throughout time on a single mouse, without having to sacrifice mice at multiple time points during the experiment. We use the optimum procedures to reduce the number of mice. We always aim to maximise the amount of data we get from each 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 breast cancer, it is important to study it in the context of a mammary gland. Therefore, the most accurate cancer models are in mammals. Mice will be used because their physiology is well studied and husbandry and experimental techniques are already established and optimized. Severe combined immune deficient (SCID) and nude mice allow the use of xenografts and can be employed to study the biology of human malignancies. Animal suffering will be minimised by making every effort to keep the tumour models employed at the subclinical levels. The generation of experimental tumours with fluorescently labelled cells coupled with microscopic analysis of tissues enables the detection of small metastasis. This reduces the overall tumour burden in the primary site needed to detect metastasis. Similar benefits are expected from the use of non-invasive fluorescent or bio-luminescent imaging

<table>
<tr><td>Project 24</td><td>Assessment of tumour initiation and metastatic spread</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cancer, Metastasis, Drugs, Endocrine Resistance</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>Yes</td><td></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></td><td>No</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 primary objectives of our translational research programme are to discover and validate new molecular targets and small molecules for anticancer drug development. New chemical entities are initially selected on the basis of in vitro (experiments performed in laboratory tubes) and cellular efficacy. Before any lead compounds can be considered for clinical development, it is first necessary to determine their efficacy in vivo (experiments performed using animal models) using appropriate mammary tumour models. Specifically, the clinical potential of new drugs will be assessed by their capacity to inhibit the growth of human tumour xenograft (transplantation of human cancer cells in immunocompromised mice).</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 work is to evaluate new cancer targets and therapeutic agents. These findings will (a) confirm the importance of these targets and (b) develop new therapeutics that will, in particular, prevent or reverse drug resistance in cancer (c) demonstrate the utility/efficacy of new cancer drugs and (d) develop therapeutics jointly with appropriate companion diagnostic(s) to enable use in personalised medicine</td></tr>
</table>

	setting. The results obtained will be used to drive cancer treatment through clinical development of the therapeutic targets and drugs, ultimately towards the evaluation of successful targets/drugs in Phase I/II setting. Moreover, assuming successful proof of principle, we would expect to publish these results in suitable academic peer reviewed journals.
What species and approximate numbers of animals do you expect to use over what period of time?	Species: Mouse. Approximate number of mice: ~2,900 for 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?	Level of severity: Moderate Animals exhibiting any two of the following signs: weakness, hunched appearance, piloerection, diarrhoea, vocalisation and struggling when handled, lack of mobility for >1 hr or increased resting breathing rate will be killed by schedule 1 method or exsanguinated by cardiac puncture under terminal anaesthesia. Animal with excessively laboured breathing (respiratory distress) or > 20% weight loss with or without other signs will be killed immediately by schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Development of new anticancer drugs requires evaluation of efficacy in animal tumour models prior to clinical trials. *In vitro* assays such as cell based screening cannot display *in vivo* efficacy of lead molecules. Therefore, *in vivo* work is needed to determine the therapeutic potential of lead molecules. The rodent xenograft models are widely regarded as the most appropriate and least severe to evaluate new anticancer drugs. Nevertheless, *in vivo* assessment is only carried out following rigorous testing of potential targets and/or drugs using *in vitro* assays and in cell culture model systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The following measures have been taken to reduce the number of animals: • Use of *in silico* (prediction of data using a computer software program) and *in vitro* assays in the lead selection and optimisation phase. • Tumour cell lines are stored in liquid nitrogen when

	not in use as its own control reducing • overall the number of animals to be used. • A statistician has been consulted in designing our studies for advice on the minimum number of animals required to ensure 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.	*Choice of species:* In-bred mice and rats will be used to reduce experimental variance; majority of the work (~95%) will be in mice but on occasion we may require the larger rat model to enable visualisation of lesions or to obtain more blood for a pharmacokinetic study. Mice and rats will be used in the studies because they are the lowest animals on the evolutionary tree for which suitable models of cancer are available. In about 90% of cases we will use immune-deficient animals for tumour induction since these will give us the opportunity to study the biology of human tumours without rejection. Nude and SCID mice will be maintained in individual ventilated cages (IVC) to avoid infections. Syngeneic tumours will also be implanted subcutaneous on the back or flank and as such will have minimal effects on the movements of the animals. The association of imaging signal with direct enzyme expression/ activity will permit clinical translation of the imaging paradigm. In addition to the more conventional subcutaneous tumour model we will, in a few cases (<5%), also assess imaging agent uptake in orthotopic, metastatic or transgenic animals. These animals are appropriate for studies of drug resistance. *Animals welfare:* All animals undergoing recovery surgery will receive routine analgesia such as Carpofen or Meloxican by injection or in drinking water as advised by the NVS. Analgesia will be administered for at least 1 day for minor surgery and at least 2 day for major surgery. After a schedule 1 kill, the tumour would be excised for histological examination. This approach should fit the study's scientific needs while not inflicting undue distress on the mice used. *Adverse effect monitoring:* Subcutaneous tumours will be visualised by eye and measured by callipers. Haematological cancers, othotopic tumours and tumours growing in transgenic animals will be monitored by imaging. Tumour size will be monitored to ensure the animals do not experience any discomfort. The size of tumour that we will be using in this project are unlikely to affect the health of the animals. Adverse effects due to drug administration will be monitored in accordance with NCRI guidelines. For any adverse effects, we will

	monitor body weight in the same way as a drug treatment. To find out the maximum tolerated, effect dose of a treatment, a limited number of mice are used in an escalating dose design. Although this entails more interventions and potentially adverse effects for those mice, we believe that the potential adverse effects are easy to recognise. All protocols will be mild or moderate. Detailed information concerning tumour and animal behaviour under experimental conditions will be recorded and shared with other researchers (as appropriate). We will monitor animals regularly in accordance with NCRI guidelines for the welfare of animals in experimental neoplasia and in accordance with UK Laboratory Animal Science Association (LASA) good practice guidelines (as discussed in Workman P et al. 2010).

Project 25	**RUNX1 functions in normal tissues and cancers**	
Key Words (max. 5 words)	Haematopoiesis, leukaemia, cancer, reprogramming	
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)	The overall aim of this project licence is to investigate the expression and function of RUNX1, a gene frequently found altered in leukaemia and solid tumours, and of RUNX1 associated genes, in normal and malignant haematological organs and epithelial tissues.	
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)?	Around one in four of all deaths in the UK are caused by cancer highlighting the urgent need for improved cancer treatments. This project will provide insights in the role of RUNX1, a master regulator of blood development frequently mutated in leukaemia and solid tumours, and of RUNX1 associated genes in blood development, maintenance, haematological and epithelial cancers. Our findings will be published in peer reviewed journals and will be of interest to clinical and academic scientists. By understanding these mechanisms, we will be able to identify new candidate therapeutic targets and develop potential new therapeutic strategies which can be translated into the clinic	
What species and approximate numbers of animals do you expect to use over what period of time?	Mice will be used in this project. We anticipate using around 4,000 animals during the 5-year period 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?	Most procedures in the protocol, such as irradiation of animals, blood or bone marrow sampling, or injection of cells and compounds are not associated with significant side effects. Mice developing leukaemia of solid tumours exhibit signs of disease, such as hunched posture, piloerection and poor levels of socialising and interaction. Under these circumstances, and whenever else an animal displays features of ill health, or at the end of each experiment, mice will be humanely euthanized using a Home Office sanctioned method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The role of RUNX1, and RUNX1 associated genes, in normal epithelial and haematological tissues and cancers will be researched as far as possible using non-animal methods. However, there is no current non-animal system that can accurately model the homeostasis of complex haematological organs and epithelial tissues or the development of cancers in these tissues. This is because these events take place in very complex cellular architecture, involving interactions between many different cell types which cannot be reproduced in vitro. Thus without the use of a live, whole animal experimental system, the biology of haematological and epithelial homeostasis and malignancies cannot be meaningfully studied.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Use of animals will be minimised by (i) making use of in vitro model systems wherever scientifically justified, (ii) use of in vivo bioimaging to follow disease development and response in real time (rather than culling cohorts of mice at defined time points), (iii) careful experimental design informed by the expert advice of a statistician (consulted regularly) so that the minimum number of mice required to produce a scientifically acceptable result are used, (iv) the use of pilot experiments to test for the extent of an expected phenotype prior to a full scale confirmatory experiment (thus avoiding full scale experiments that may lack sufficient statistical power), (v) the use of protocols for each experiment which include the objective, proposed interventions, numbers of animals and analysis method and (vi) the cryopreservation in multiple aliquots of normal and tumoural cell populations and samples (which eliminates a requirement for continuous production of cohorts of mice).

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 have been chosen for the study because they represent the least sentient species from which meaningful experimental data can be generated, while exhibiting considerable genetic and biological similarities to humans with regard to their blood forming system. Only a mammalian model system has the potential to accurately mimic both the anatomy and complex cell biology, including microenvironmental interactions, of normal and malignant human haematological organs and epithelial tissues. Furthermore, there is considerable experience in the wider scientific community regarding the use of mice as a model system for human malignancies and many reagents exist for the phenotypic characterisation of mouse cells. The techniques used have been carefully evaluated to minimise distress to the animals. Mice used in surgical procedures will be treated with anaesthesia, analgesia and post-operative rehydration by subcutaneous injection, followed by careful observation. In other areas, irradiation doses will be administered at a level sufficient to induce bone marrow suppression but no other long term sequelae; bone marrow injections and aspirates will be not be performed routinely, only where the scientific justification is high; and in studies that result in the development of malignancy, animals will be closely monitored for health status and killed by a Home Office approved method when signs of ill health are displayed.

Project 26	**Cytokine signalling in development and disease**	
Key Words (max. 5 words)	TGF-13 superfamily signalling, transcription, cancer, embryonic development, signal transduction	
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)	Cells receive information from their environment and their neighbours in the form of extracellular signals that dictate the proteins that cells make and as a result, determine cell behaviour. This phenomenon underpins embryonic development and tissue homeostasis in adult organisms. Misregulation of any aspect of this process, whether production of the extracellular signalling proteins, the wiring of the signal transduction pathways, or the interpretation of those signals, can cause human disease, the most widespread being cancer. The overall aim of the project is to determine how extracellular ligands signal to the nucleus in normal untransformed cells and in early vertebrate development, and how these processes are perturbed in human diseases such as cancer. We will use the TGF-f superfamily signalling pathways as a paradigm. An ultimate aim is to identify targets for therapy and also prognostic or diagnostic markers for 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	The main benefits of this project is a much better understanding of the role and regulation of TGF-13 superfamily signalling in early vertebrate development and in cancer. All of this work has the potential for understanding how these pathways are misregulated	

project)?	in cancer and other diseases, which could lead to new therapies, and to the development of diagnostic and prognostic markers. We anticipate that some of our zebrafish mutants may lead to new disease models and our zebrafish and mouse transgenics will be valuable models for the research community.
What species and approximate numbers of animals do you expect to use over what period of time?	We anticipate using 40,500 zebrafish (Danio rerio) over the five-year period, together with about 2700 mice and between 500-1000 frogs (Xenopus).
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 the case of the Xenopus, we only use the adult females to produce eggs, which we fertilize with sperm from males that are humanely euthanized. The embryos are used for experiments, but are only kept until the point that they become independently feeding. We can re-use the females several times, or until they no longer produce high quality eggs. In the case of the zebrafish, our work mainly involves making reporter lines that allow us to monitor signalling by the TGF-3 superfamily members or we will mutate or delete key genes to understand the effect this has on early embryonic development. Because we will breed these fish as heterozygotes we do not anticipate any adverse effects on the adults. The embryos will only be studied for up to five days or until they can feed independently. The only procedures that the juvenile or adult fish will undergo is fin clipping for genotyping, transient warming to induce expression of transgenes and gamete harvesting. We do not anticipate adverse effects from any of these procedures and any effects that the fish do suffer are likely to be mild. In some cases, the fish may develop small tumours. Any fish showing more than moderate adverse effects will be humanely euthanised. The mice in the project will be used for tumour studies. We are trying to find out how the signalling pathways we are interested in contribute to the process of tumour formation and its spread. Some mice may be genetically altered to make them more cancer prone. Clearly, the development of tumours is an adverse effect, but this is necessary to study cancer. Most of the animals used will experience sub-threshold or mild severity procedures; we expect 20-30% to experience moderate severity procedures, such as larger tumours. Imaging will be performed under anaesthesia with animals monitored closely

	while recovering from the anaesthetic, we expect minimal adverse effects from these procedures. Once we have obtained the information we need from the experiment the mice will be humanely euthanised.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We need to use animals for several different reasons. One of our major questions is to work out how TGF-13 superfamily signalling contributes to early vertebrate development. The only way of addressing this is studying it directly in vertebrate embryos. We mostly use the zebrafish system for this, and the majority of our work on embryos is done before the animals are deemed sentient, before the onset of independent feeding. The only work that will be done on adult or juvenile fish is fin clipping for genotyping or transient warming to induce expression of transgenes. As often as possible mating will be performed followed by analysis of phenotype to determine the genotype of the fish as an alternative to fin clipping. All of the Xenopus work is done on wild type embryos before the are capable of independent feeding. With regards to the mouse work, we only perform studies on mice that cannot be done in tissue culture systems or in the Xenopus or zebrafish systems. Moreover, we will also study tumourgenicity in vitro. If our data show a good correlation between tumourigenicity in vivo and the in vitro properties of given cell lines we may be able to further limit the number of tumourgenicity experiments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	For the zebrafish work our experiments are designed to use the minimum numbers of animals necessary to attain statistically significant results: for this, there are strong practical as well as ethical incentives. Estimates of numbers of fish needed for transgenesis experiments are based on statistics on the efficiency of transgenesis from other laboratories. We will regularly review our mutant and other stocks and cull any that are no longer required. Through exchanges with other zebrafish labs, in the UK and elsewhere, we will be able to minimise the number of mutant and genetically modified strains that we keep in our own aquarium. Moreover, the stocks of adult mutant fish that we will keep will almost all be heterozygotes carrying recessive mutations and thus phenotypically normal. In addition, because we share the fish aquarium with other users, we ensure that multiple workers perform their experiments on the same day to maximise the use of the embryos from a given

	batch of fish. Any extras are fixed for subsequent experiments. For the Xenopus work we attempt to minimise our usage of animals by careful time tabling of experiments on consecutive days, with multiple in vitro fertilizations per day and multiple workers performing distinct experiments on the same day. Excess embryos are either fixed for further experiments or frozen for biochemistry. We also attempt to minimise the frequency of animal use by maintaining a healthy colony. When maintained in the correct environment, a single Xenopus laevis or tropicalis female can produce thousands of eggs. Under these circumstances the number of animals used at any one time can be kept to a minimum. For the mouse work we will try to keep as few mice as possible by careful monitoring our mouse colony and good practice. Mouse lines are routinely maintained by keeping 2-3 breeding pairs, with around 3-4 litters/year total 75-100 animals per strain/year. For crosses to enable characterisation specific phenotypes it is likely that 5-6 breeding pairs will be kept with 6-8 litters/year total 350-400 animals per strain. We anticipate maintaining up to a maximum of 3 lines at any one time. To minimise breeding, lines under sporadic use are maintained at lower levels, and frozen whenever practicable. Lines will be maintained in collaboration with other licences wherever possible to minimise redundant breeding. For the tumour induction assays we will use statistical tests to determine the appropriate number of mice required to test a hypothesis with statistical meaningful results. We will aim at all times to maximise the amount of data we get from each 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 the mouse model where we want to investigate tumourgenesis in a mammal. We use the zebrafish model for the vertebrate developmental work, as these embryos develop ex utero and can be manipulated genetically and are transparent, and thus ideal for imaging. We use the Xenopus embryos, for developmental work, where we might want to do cell or tissue transplants or disection, as they are ideal for this. Having the Xenopus system in combination with the zebrafish allows us to test the generality of our results in vertebrates. All embryological work is preceded by in vitro or

	tissue culture studies to test the approach or activity of the biological entity to be introduced into the embryo. Failure at this preliminary stage is taken as final and no in vivo work will take place until this step is successful. In addition, for the zebrafish we will minimise suffering by taking pains to maintain the general health of the fish population, by attention to water quality, feeding regimes, and fish population density in each tank. We will check all breeding stock daily and cull any that show signs of significant illness or deformity. Where surgical or other potentially distressing procedures are required, eg fin clipping, we will perform them under general anaesthesia. Any fish or fish larvae showing signs of distress on recovery from a surgical or other procedure will be killed promptly by an approved method. For the mouse work we will also check the animals every day and kill any exhibiting signs of significant illness. We will also reduce the overall tumour burden on mice and thus minimise possible adverse effects, by for example using the non-invasive imaging for metastasis assays.

Project 27	**NKT cells and related immunity in health & disease**	
Key Words (max. 5 words)	Cancer obesity NKT (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)	Tumour-bearing mice will be compared for animmune white blood cell subset, called Natural Killer T cells" ('NKT') cells and for tumour growth on standard and high fat diets to test the hypothesis that obesity-induced immune decline contributes to weakened anti-tumour responses. Interventions to reverse immune decline will be compared on tumour-bearing mice on standard and high fat diets to determine specifically whether reversing NKT defects aids anti-tumour responses. NKT cells are a specialised immune cell type that can enhance anti-tumour immune responses. These cells can be activated by certain stimuli and other agents and vaccines, which may be able to stimulate anti-tumour immunity. This proposal willassess the ability of several such approaches to enhance immune responses to cancer, and determine how the responses to the best treatment can be further optimized. The goal will be to translate these results into a clinical trial taking into account cancer with and without obesity.	
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 purpose of the proposed research is evaluating novel immunotherapy for cancer and specifically, to determine if these approaches and strategies that manipulate immunity can eliminate tumours and if so, how. Modulating the immune system is a way to	

project)?	circumvent immune tolerance to tumour associated antigens, a major clinical problem, which will be attempted to treat cancer in mice. The experimental design and findings emanating from this work will allow for a similar design in treating human cancer in the future.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, up to 600 / year including breeding and experimental, x 5 years = 3000 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?	Progressive tumour growth will occur in unprotected mice which have not been protected by the therapies being tested (if that occurs) and in the control groups, but is likely less in the majority of the mice, since most are treated groups, based on previous results in other models. Tumour growth leads to moderate level distress, but death is not an endpoint and mice with progressively growing tumours will be euthanized once the largest tumour reaches a maximal size of 1 cm in any dimension. Also, regular monitoring and sacrifice of any ulcerating tumour-bearing or otherwise sick mice will minimise discomfort. Treatments will not induce or add to discomfort. All mice will be humanely euthanized before developing severe illness or by 1 year of age, if sooner.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Only live mice express the complete immune system and in transgenic tumour models, the tumour and immune system co-evolve, as in patients. Finally, one can only compare potential treatment strategies in mice, since there are no reliable tissue culture surrogates for immune antitumour responses to immunotherapies. Fortunately, however, the simplest feasible mammalian model, mice, provide all the advantages of similar physiology as well as well characterised reagents (e.g. antibodies to cells and proteins) and tumour models are available and mechanistic insights from genetic models can be derived along with the ability to manipulate to determine how therapies work in a lower and non-endangered species.
2. Reduction Explain how you will assure the use of minimum numbers	By comparing all treatments in parallel, the same controls will be used for all, thus reducing numbers from equal numbers of controls as treated mice to 40

of animals	% fewer mice in total. Published previous results in other tumour models and individual therapies indicates relatively small group sizes will be sufficient for clear results. As in clinical trials, once it becomes clear which approach(es) provide the best therapeutic effects, other approaches will be dropped and remaining control mice switched to the optimal means or sacrificed humanely.
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 have fundamentally similar immune systems to humans, including the NKT cells to be exploited in novel immunotherapeutic approaches. Thus there is no need for higher mammals, including primates, in this pre-clinical study. The tumour model to be employed is a relevant transgenic one (therefore close to human cancer) and directly relevant to the most likely clinical trials to follow. Previous such mouse studies have been accepted as sufficient for initiating a clinical trial of NKT cell reconstitution. This approach showed encouraging immune and tumour responses in a major medical centre setting, but is not practical at large scale in non-teaching hospital environments and further approaches which have been identified have wider and potentially greater therapeutic potential. Therefore, it is proposed to compare available NKT cell-based immunotherapy approaches together in the most efficient head to head comparison possible (based on limited tumour growth and immune studies, *not* survival), to provide pre-clinical data to support further clinical trials. In summary, these non-survival studies will employ our optimal protocols, maximising use of the 3 Rs.

Project 28	**Signalling pathways in cancer**	
Key Words (max. 5 words)	Cancer, protein kinase, protein phosphatase	
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
		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 generate animals lacking regulators of protein kinase signalling and utilise these animals to study the roles of these proteins in the genesis and progression of cancer using defined transgenic models.	
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 work will reveal new information about the regulation of protein kinase signalling in cancer development. This information will be of interest to both basic and clinical scientists working in the field of cancer biology. In the longer term, our studies may identify novel drug targets or suggest strategies for the treatment and/or management of cancer.	
What species and approximate numbers of animals do you expect to use over what period of time?	Mice (15,000) over the five-year term 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?	The animals would be expected to develop tumours either externally (skin) or of internal organs (lung, pancreas or intestine). The majority of these outcomes will be of mild-moderate severity and animals will be killed and analysed at the conclusion of each defined experimental protocol. Animals could suffer from signs seen in these tumour types in humans such as dyspnoea, jaundice, neurological	

	signs, behavioural disturbances or weight loss. In all cases, animals will be closely monitored to ensure that signs will be picked up early and the animals killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Wherever possible, cultured cells *in vitro* will be used to gain functional information. However, complex physiological and pathological processes such as tumour initiation, progression and metastasis cannot be studied in cultured cells. Furthermore, while there are technologies available that allow loss of gene function studies to be performed, such as the use of siRNA knockdown, these are restricted to use in cultured cells.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experimental design will be kept as simple as practically possible, in order to maximise the information obtained from the minimum number of animals. Pilot studies will be carried out with no more than 5-10 animals per group in the first instance in order to assess the potential magnitude of the experimental response. The exact numbers of animals required will vary depending on genetic background and disease model. Our experimental designs have been discussed with the local statistical service (University of Dundee) and advice concerning the minimum number of experimental animals required to allow a sufficiently powerful statistical analysis obtained.
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 only mammal in which transgenic and knockout technology works reliably and there now exists a formidable array of mouse mutant and transgenic strains, which accurately model many human diseases. In the case of cancer, recently developed systems for the tissue-specific expression of activated oncogenes such as Kras and Braf have facilitated the development of mouse models, which accurately reflect the incidence and natural history of tumours of the lung, pancreas and intestine. In addition, well-established protocols of chemical carcinogenesis can be utilised to study tumours in the skin, liver and lungs of transgenic animals. During experiments designed to determine the influence of gene loss on the development of tumours, animals will be monitored carefully for signs of ill health such as dyspnoea, jaundice, neurological signs, behavioural disturbances or weight loss. In all cases, animals will be closely monitored to ensure tumour

	burden does not interfere with normal functions and any animal experiencing unexpected pain, distress or deterioration in its general condition will be killed by a Schedule 1 method. All animal work will follow current guidelines for the welfare of animals used in cancer research.

Project 29	**Mouse models of Prostate cancer initiation & progression**	
Key Words (max. 5 words)	Cancer, prostate, tumour-initiating cells, metastasis, 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)	Our aim is to improve the understanding of prostate cancer initiation, progression and metastasis. Ultimately, by the use of our mouse models, aim to develop new treatment strategies that can be used in the clinic.	
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 prostate tumour initiation and progression to secondary cancer (metastasis) mechanisms. Moreover, by understanding these mechanisms we will be able to tackle them in a therapeutic perspective; these animal models will provide more powerful methods to elucidate the underlying cellular and molecular mechanisms of tumour progression and metastasis to pursue novel targeted treatments to individual cancer patients. In addition, by assessing standard-of-care and new hypothesis-driven treatment approaches we aim to rationalise therapeutic decisions in the clinic based on the results from our studies. Finally, we expect to publish our work in peer-reviewed journals thus sharing our findings with the scientific community.	
What species and	Mice, around 5500	

approximate numbers of animals do you expect to use over what 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 level of severity? What will happen to the animals at the end?	Normal and genetically altered mice will be used to investigate the role of certain specific molecular pathways in the induction, progression and metastatic potential of prostate cancer cells. The animals will have tumours grown in them initiated through either use of cancer prone genetically modified mice; by the use of inducing agents or by the implantation / injection of existing tumour cells / tumour pieces. To mimic some of the testosterone driven prostate tumour processes, some of the mice will, on occasions be castrated, under general anaesthesia. Other surgical procedures requiring general anaesthesia and analgesia are tumour implantation (into specific organs of interest), tumour biopsy (a gold standard method used clinically) and the implantation of small drug delivery systems under the skin. To monitor the tumour growth, a combination of methods will be used: the use of callipers (for subcutaneous implanted tumours) and imaging under light general anaesthesia will be performed on a number of occasions to monitor tumour growth. On occasions it will also be necessary to inject, by one of several possible different routes, chemical agents that will allow better imaging of the internal tumours. 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 (i.e. 20% loss in bodyweight) and the mice culled humanely. 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 of the experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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. The majority of our current understanding of cancer induction comes from the “test tube” analysis of late-stage tumour tissue removed from cancer patients. A collection of “test tube” assays is performed to understand important

	points of tumour biology. While this has elucidated many changes in cancer cells, it provides little information about the local environmental factors influencing early-stage cancer development in life. Also certain hallmarks of cancer, such as spread of cancer and blood vessel formation, are impossible to study in “test tubes”. Therefore, mouse models are important for studying the in life aspects of human cancer development. Mouse models have been engineered to develop cancers, which accurately mimic their human counterparts, and have potential applications to test the effectiveness of novel cancer therapeutics. This cannot be replaced by “test tube” studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our use of test tube methods limits the number of animals required for the in life investigation stage. For our engineered models an efficient breeding strategy will minimise the number of mice used to obtain the desired genotype. The proposed experimental designs and methods of analysis of the results are advised by statistical guidelines and a bioinformatician scientist to provide meaningful data minimizing the number of animals used in each experiment. The planning of individual experiments will involve designs that maximise the information obtained from the minimum 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.	Mouse models faithfully recapitulate the human disease. Moreover, the mouse genes share 98% similarity with human genes. Husbandry and procedures are used that minimise actual or potential pain, suffering, distress or lasting harm and/or improve animal welfare in situations where the use of animals is unavoidable. Animals will be group housed in an enriched environment to reduce stress where possible. Anaesthetic and analgesic regimes as well as appropriate humane methods of culling will be used. Lowest practical blood volumes will be taken for analysis. We ensure no visualisation of procedures in other animals and transport arrangements between facilities in appropriate containers.

Project 30	**Characterisation of cellular heterogeneity in ovarian cancer**	
Key Words (max. 5 words)	Stem cells, xenotransplantation, cancer, lineage-tracing	
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 main focus of this project proposal is to identify and characterise the different types of cells that make up the normal fallopian tube epithelium in mice, and in human ovarian tumours. We are particularly interested in identifying which cells function as the stem (mother) cells, and determining what types of daughter cells they generate. Understanding these parent-progeny relationships are very insightful because evidence has emerged that these relationships are often maintained in cancer, and may explain the behaviour of tumours and the emergence of therapy resistance. A hypothesis that we propose to test is whether or not the more mature daughter cells are sensitive to therapy, and thus killing of these cells can shrink the tumour, but the more primitive mother cells (e.g., the cancer stem cells) are resistant to the therapy, and they proliferate and cause the tumour to regrow. If this hypothesis proves to be correct, then identification of the molecular pathways of these therapy-resistant mother cells would help in the design of a treatment that could complement existing therapies.	
What are the potential benefits likely to derive from this	Identification of molecular pathways that drive cancer growth. These pathways, once identified, could be	

project (how science could be advanced or humans or animals could benefit from the project)?	targeted using exiting or yet to be developed drugs.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use approximately 16,000 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?	Some of the animals that we propose to use will be used as hosts for growing human ovarian tumour cells. We use surgical techniques to implant these tumours under the skin in the flanks of the mice. Our experience has shown that almost 100% of mice will recover from these surgeries with no adverse effects. Rare problems, when they do occur, are usually associated with wound closure after surgery, although these normally heal on their own. A problem that is expected to occur at a low frequency (<10% of mice), is the ulceration and scabbing of the skin above the tumour. It is expected that in most of these cases, the skin is expected to heal within a few days. In some cases, the ulceration will progress, and these mice will have to be monitored closely to prevent this exceeding a moderate degree of severity. Other mice used in experimental procedures will be injected or fed nucleosides, which are molecules that are incorporated into the DNA of dividing cells. This is used as a method to track cell division in mice. Other than the transient discomfort of an injection, these mice are not expected to experience any adverse effects. Some of the mice will be used to maintain breeding colonies. We don't expect the animals used for this to experience any adverse effects. All animals at the end of the experiments will be killed in a humane fashion.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In vitro methods that promote the growth of normal and fallopian tube cells and maintain them in a state that mirrors the in vivo state have not been developed. As a result, transplantation/analysis of cells into mice still remains the only method of growing and maintaining tissue in its natural state.
2. Reduction	We reduce the number of mice used by a variety of

Explain how you will assure the use of minimum numbers of animals	methods: 1. Using appropriate experimental design (e.g., transplanting appropriate numbers of cells such that engraftments give useful information, and performing statistical tests in advance in order to identify the minimal number of mice required for the experiment) 2. Transplanting two grafts per mouse. Transplanting multiple grafts in a mouse often does not increase the severity of the adverse effects that mouse will experience, but it can reduce the number of mice required by 50%.
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.	Only specific genetically modified mice can be used for growing human tumours since only these animals are immune-deficient enough to permit tumour growth without immune-rejection. We have spent considerable time in the past working with the veterinary surgeon to optimise our surgical techniques. The vast majority of our mice recover from surgery without any complications. As discussed above, we have selected the least invasive method (injection underneath the skin) for growing tumours in mice. We prefer this site because it is easy to monitor tumour growth, it is not an invasive procedure and there is less risk of compromising the function of internal organs. In all experiments, we practice good surgical techniques and we work with the animal facility staff (and when required, the veterinary surgeon) to closely monitor mice that have had surgery or have undergone other regulated procedures. Mice that display a level of adverse effects that reach a predefined level are killed in a humane fashion.

Project 31	**Mouse models of KRAS-driven cancer progression & therapy.**	
Key Words (max. 5 words)	Lung cancer, oncogene, metastasis, 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)	Our aim is to improve the understanding of lung cancer initiation, progression and metastasis. Ultimately, by the use of our mouse models, we aim to develop new treatment strategies which can be translated into the clinic.	
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 lung tumour growth and spreading mechanisms. Moreover, by understanding these mechanisms will allow them to be tackled in a therapeutic perspective; these animal models will provide more powerful methods to elucidate the underlying mechanisms of tumour growth and spread, and introduce drugs targeted to individual cancer patients. In addition by assessing standard-of-care and new hypothesis-driven treatment approaches the project aims to rationalise therapeutic decisions in the clinic based on the in vivo results from our studies. Finally, the work will be published in peer reviewed journals thus sharing our findings with the scientific community.	
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, around 5600 5 years	
In the context of what you propose to do to the animals, what are the expected adverse	The mice will have tumours grown in them initiated through either use of cancer prone genetically modified mice or by the implantation / injection of	

effects and the likely/expected level of severity? What will happen to the animals at the end?	existing tumour cells / tumour pieces. Other surgical procedures requiring general anaesthesia and analgesia are tumour implantation (into specific organs of interest), primary tumour removal (during the study of secondary cancers). To monitor the tumour growth, a combination of methods will be used: the use of callipers (for subcutaneous implanted tumours) and imaging under light general anaesthesia will be performed on a number of occasions to monitor tumour growth. On occasions it will also be necessary to inject, by one of several possible different routes, chemical agents that will allow better imaging of the internal tumours. Therapeutic agents, both clinical and experimental will be administered either orally or by injection. The vast majority of mice are not expected to show any sign 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	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The majority of our current understanding of carcinogenesis comes from the test tube analysis of late-stage tumour tissue removed from cancer patients. Our lab performs a collection of test tube assays to understand important points of tumour biology. We use these test tube studies to identify new mechanisms involved in different stages of lung cancer. While this has elucidated many changes experienced by cancer cells, it provides little information about the factors influencing early-stage cancer development in the body. Cancer development is dependent not only on the changes occurring within the transformed cells, but also on the interactions of the cells with their normal cells surrounding the tumour. 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 life aspects of human cancer development. Transgenic mouse models have been engineered to develop cancers, which accurately mimic their human counterparts, and have potential applications to test the

	effectiveness of novel cancer therapeutics. This cannot be replaced by test tube studies or different in-vivo models such as insects. Lung cancer can only be effectively studied in animals with lungs, so ruling out insects and fishes.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our use of in-vitro methods limits the number of animals required for the in-vivo investigation stage. 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.
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. 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 ensure no visualisation of procedures in other mice and transport arrangements between facilities in appropriate carrying boxes. We will use non-invasive imaging procedures to follow lung cancer and metastatic tumours. When the scientific endpoint has been reached the animals will be killed before any humane end-point is reached.

Project 32	**Inflammation and Viral Tumourigenesis**	
Key Words (max. 5 words)	EBV, cancer, chronic inflammation, anti-oxidant	
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)	Specific viruses can lead to cancer formation and work in this project involves one such virus: Epstein-Barr virus (EBV). It is known that many viruses cause inflammation, an immune response by the body which has evolved to help expunge the virus. However, in the “arms race” of virus against host, EBV has evolved not only to avoid elimination from the host, but also to use immune factors to promote its own survival and propagation. Chronic inflammation is also known to be a cancer risk. We have previously identified one of the viral genes that is responsible for inducing chronic inflammation. What is not known is, if this state contributes to EBV associated cancer, which will be under study in this project. Furthermore, the molecular pathways by which viral genes increase the risk of cancer are not fully understood and are under investigation in this project. This knowledge will be used to design potential ant-viral/anti-cancer therapeutics. Under certain conditions (such as the rapid growth of cancerous cells), molecules containing oxygen, that are highly reactive and can be damaging to DNA and tissues, are over produced. These are termed reactive oxygen species (ROS) and when more than is normal are produced, the cells or tissue are regarded as being under oxidative stress. There is a	

	cyclic link between oxidative stress and chronic inflammation. Oxidative stress can lead to chronic inflammation and inflammatory cells produce ROS. Thus, if the inflammation remains unresolved, the state becomes progressively worse. Hence there is a huge pharmaceutical and dietary supplement market to supply “anti-oxidants”, to both combat chronic inflammation and potentially cancer. However, the effects of long term anti-oxidant treatment and whether such treatment can combat cancer onset or progression, is unknown. Therefore the objectives in this project are to further determine the links between EBV associated cancer and chronic inflammation; and between oxidative stress, chronic inflammation and 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)?	Tens of thousands of people each year develop an EBV-associated cancer. Currently, only standard chemotherapy or radiotherapy options exist for these patients. A better understanding of the action of viral genes *in vivo* will permit targeted therapies to be developed and tested in the genetically altered models described in this project. Millions people world-wide take dietary supplements of anti-oxidants in the belief that this will reduce the risk of cancer, however, scientific evidence for this is currently lacking. The more *in vivo* data collected on this topic, the more accurate the evaluation of the value of dietary anti-oxidants and thus this work will advance our understanding of anti-oxidants and cancer, to human benefit.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse, maximally 4,000 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?	In certain genetically altered mouse models, the mice will develop inflamed skin (particularly on the ears), which might be itchy or irritating (mild severity). In other models, the mice will develop tumours, usually on the skin or alternatively lymphoid tumours (lymphoma). The majority of the skin tumours are benign (wart-like) and are expected to be small and have no significant impact on the animals’ general well-being (mild severity). Larger tumours on the skin may ulcerate or cause discomfort, which is visually evident (moderate severity). Similarly, lymphomas become evident as an abdominal swelling before the

	mouse displays signs of discomfort. Any mouse showing signs of suffering will be humanely killed without delay. All mice are humanely killed at the end of the study period.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The complex interplay between the immune system and cancer cannot be effectively recapitulated *in vitro* or cell culture systems. Similarly, the diverse action and consequences of antioxidant treatment in the body, cannot be determined in culture experiments. Moreover, all new therapeutic regimes and medicines benefit from being tested in an *in vivo* model system before translation to human trials. As such, non-animal alternatives for the described objectives either do not currently exist or don't fulfil the objective.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In all protocols the numbers of mice required to generate a statistically significant and/or otherwise meaningful result are considered carefully in advance of the experiment (and based on power calculations and/or considerable experience) and numbers of mice used do not exceed this. The use of *in vivo* imaging for certain studies (particularly time courses) reduces then numbers of mice required on study compared to conventional assays.
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 provide an excellent model for human disease, particularly diseases of the immune system and cancer, for several reasons. They are highly genetically similar to humans, suffer from the same range of diseases and have an almost identical immune system to humans. As such, determining gene function and immune responses in mice has proved to be hugely informative with regard to human disease. We will only use well established reagents and protocols to study the mice. In all protocols, the procedures which cause least pain and distress in achieving the objective are followed. In all cases the mice will be closely monitored and any mouse showing signs of distress during the procedure will be humanely killed.

Project 33	**Mouse Models of Human Cancer**	
Key Words (max. 5 words)	Cancer, GEMs, therapy, transplantation.	
Expected duration of the project (yrs)	Five	
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 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	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	

animals could benefit from the project)?	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 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 16,000 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. 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. A proportion of animals (no more than 25%) 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 (15-20%) will be given anti-cancer treatments, changes in their diet 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 34	**Investigating epithelial cancer in vivo**	
Key Words (max. 5 words)	Colorectal Cancer, Pancreatic 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)	Epithelial cells are those that line the skin and most internal organs such as gut, breast and lung. Epithelial cancers therefore are the most common cancers and are the most frequent causes of cancer morbidity. Moreover many of these cancers respond poorly to current therapeutic intervention especially if diagnosed at advanced stages (eg pancreatic, bowel, gastric and liver cancers). Our objectives are to: 1. Learn more about the mutations that drive cancer 2. Find new ways of targeting cells that carry these mutations 3. Assess if we can activate immune cells to target 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)?	We hope to uncover and test new targets for development of medicinal therapies for the treatment of late stage epithelial cancer. We hope to learn more about the early stages of cancer that could provide important clues to prevention of cancer.	
What species and approximate numbers of animals do you expect to use	We expect to use up to 25,000 mice per year over 5 years for this project. Around 50% of these will not undergo any scientific procedures, but will be used	

over what period of time?	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 show predisposition to epithelial cancer or will receive a transplant of tumour tissue or cells from mouse or human cancer. Approximately 50% of the mice will not show any adverse effects related to the breeding and not undergo any procedures except for ear notching for identification and genetic testing. These will be kept in normal housing and humanely killed when they are no longer needed for breeding. We will often be able to use tissue samples from these mice after they are killed as normal controls. Some proportion of the animals (approximately 20%) will be predisposed to cancer and will be monitored carefully for clinical symptoms. Symptoms include paling of feet, anaemia, 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 euthanised.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Epithelial cancers are very complex cancers that involves a number of different cell types e.g. cancer cells, immune cells, blood vessels. In addition, epithelial tumour cells grow within a particularly dense matrix that plays a major role in tumour development, growth and spread, and stops drugs from working properly. Current non-animal models cannot reproduce this situation and are not appropriate for studies to understanding epithelial cancer progression or for testing of new drugs. This is particularly relevant for immunotherapy approaches where the patient's own immune system needs to be reactivated to kill the tumour.

2. Reduction Explain how you will assure the use of minimum numbers of animals	We perform pilot 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 primary intestinal cell lines *in vitro* or fly intestines so that only our strongest hypotheses are tested in vivo.
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 genetic models of cancer are widely accepted to be the most closely representative of human cancers. The tumour forms in the correct tissue and spreads via the normal routes and the tumours often progress through the same stages of pre-cancer as in humans. We use state-of-the art genetic models to ensure that the cancer develops in the correct organ/tissues and there are as few side effects as possible due to breeding or treatments. This is done using mice that are engineered to produce enzymes, specifically in the target issue of interest, which can cut out or activate genes. Animals will receive anaesthetic and/or analgesic treatments where appropriate. All animals will be monitored regularly for signs of normal behaviour and will be humanely killed if they exhibit moderate adverse signs.

Project 35	**Cancer imaging and drug development**	
Key Words (max. 5 words)	Cancer, imaging, diagnosis, early response, drug development	
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)	The main objective of this project is to develop new imaging agents and therapeutics for cancer. An important translational strategy is the development of 'companion imaging diagnostics' for expediting the development of novel therapeutics. Cancer is a disease of major unmet medical need (1 in 3 to 1 in 2 people are affected) and potential benefits of research into the diagnosis and treatment are clear. Targeting properties of cancer cells that govern growth and metastasis for tumour diagnosis and therapy underpins our discovery efforts. We will design imaging agents to image important characteristics of cancer. Because current drugs are ineffective in most cancers, new drugs, particularly those that can overcome drug resistance, are needed. We are exploiting our evolving understanding of the molecular pathology of cancer to develop new drugs. The overall plan is to identify, screen and validate imaging agents and drugs for diagnosis and therapy. A significant part of the work will be done using isolated cancer cells grown in the laboratory in cell culture dishes. These initial experiments will ascertain relevance of the processes being studied, and permit optimisation. Ultimately, however, animals have to be used to determine whether in the whole organism, the specific biological	

	target is being modulated. Mice and rats will be used because they are the lowest animals in the evolutionary tree for which suitable models of cancer are available. We will use immune-deficient animals for tumour induction since these will give us the opportunity to study the biology of human tumours. An average of 2190 rodents could be used annually in this project.
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)?	i. Understand the biology of cancer in living subjects. ii. Develop new diagnostics for cancer. iii. Develop new methods indicating very early whether patients are resistant to drug treatment saving patients cumulative toxicity and switch to other drugs. iv. Develop new drugs that could overcome drug resistance.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice and Rats (95% will be mice) Average of 2190 animals per year for 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?	Most animals will undergo procedures involving mild discomfort a pin-prick and non-invasive imaging. Animals will be anaesthetised for this procedure. Animals may receive therapeutics or develop tumours, procedures that could potentially cause moderate discomfort. Animals will be monitored closely including use of image-guided monitoring of tumour size. Serial sampling by imaging is both better science and reduces animal use. Animals will be housed with appropriate bedding, nestling material and with cage toys for a stimulating cage environment. For immune deficient/transgenic animals, individually-ventilated cages will be used to avoid infection. We will monitor animals in accordance with National Cancer Research Institute guidelines for the welfare of animals in experimental neoplasia, as well as UK Laboratory Animal Science Association good practice guidelines (LASA guidance). At the end of the study, animals will be humanely killed or euthanised. In summary, we aim to develop imaging methodologies and therapeutics that will benefit science, animals and people. We consider the overall severity as moderate.
Application of the 3Rs	
1. Replacement	Several in vitro models will be used however we also

State why you need to use animals and why you cannot use non-animal alternatives	need to use animals. • Imaging contrast cannot be determined by any other way. • Targeted drug action cannot be fully evaluated in other models • Companion diagnostic paradigms cannot be developed in other models
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our study design assures reduction • Use of longitudinal imaging to allow the same animal to be used as their own control. • Obtaining high content data (in tumour and normal tissues) from each animal than will be obtained from sacrificing animals at multiple timepoints. • Robust (clear, easy to read, does not impact welfare) animal identification
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.	Our study design assures refinement • Use of anaesthesia • Use of analgesia • Environmental enrichment • Non-surgical methods of biologic endpoint detection on multiple occasion by imaging • Earlier disease endpoint detection by imaging which is less severe than clinical endpoint • Use of rodents instead of higher order animal species and use of the least sever tumour model (largely subcutaneous); strict limits to avoid toxicity. The mouse will be used in the majority of cases but on occasion the larger rat model will be used to enable better visualisation of lesions or to obtain more blood for analysis. • Researchers will be seek advice regarding care and well-being of animals from NVS and NACWO as necessary, and take the necessary endpoint steps if advised to do so to avoid suffering.

Project 36	**p53-family in physiology and cancer**	
Key Words (max. 5 words)	Cancer, aging, development, metabolism	
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
		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 p53-family (including p53, p63 and p73) is considered among the most powerful family of genes for human physiology and disease. These genes play significant roles in biological processes, such as embryonic development, aging, metabolism and cancer. In particular p53 is one of the major players in tumour suppression. It is the most mutated gene across all human cancers (more than 50%) and its alteration is associated with highly aggressive tumours and poor survival expectancy. p73 is importantly involved in regulation of neuronal development. Alterations of p73 are also associated with neurodegenerative disease, such as Alzheimer's disease. p63 is a master regulator of epidermal development and familiar mutations of this gene are associated with hereditary epidermal and ectodermal dysplasia. Altered expression of p63 and p73 can also be identified in cancer.	
What are the potential benefits likely to derive from this project (how science could be	Currently, the most effective way of investigating the function of a given gene at the level of the whole animal is through the generation of genetically altered	

advanced or humans or animals could benefit from the project)?	mice. Identification of the exact functions and regulations of p53 family members as well as their interactions with others genes is urgently required to understand the molecular basis of human diseases and to develop alternatives and innovative therapeutic approaches.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use less than 10,000 mice in the next 5 years. This will include up to 10 different colonies of genetically modified mice for the different isoforms of p53 family members and some more colonies of genetically modified mice for interactors of p53 family members.
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 our research program the severity will be limited to a moderate level. In any procedures, adverse effects will be minimised by carefully monitoring the animals for clinical signs. No animal will be allowed to show any severe clinical sign and animals with a number of moderate clinical signs will be culled.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	These studies require the use of whole tissues and systems from an intact organism, which would not be possible in, for example, a cell culture system due to the requirement for cross-talk between different organs and tissues.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Before performing any approach on animals, all possible in-vitro experiments will be carried out to target the animal experiments to highest probability of success. Every experiment will be performed using the minimum number of animals required to obtain statistically significant 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.	Currently, the mouse is the most widely used species in the international research community for studies of the in-vivo functions of mammalian genes and is the only one for which many elaborated transgenic techniques are available. Careful experimental planning and long-term storage of biological samples (blood, serum, cell lines, tissues, etc) will help reduce the use of animals. In all procedures, adverse effects will be minimised by carefully monitoring the animals for clinical signs. No animal will be allowed to show any severe clinical sign and animals with a number of moderate clinical signs will be culled.

Project 37	**Novel functions of p53 mutations in tumour progression**	
Key Words (max. 5 words)	Tumours, mutant p53, RCP, engulfment	
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
		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)	Mutations in p53 have been associated with more aggressive cancers and are often difficult to treat as a result of resistance against cancer therapeutics. This research aims to prove that a certain molecule (RCP) is involved in this resistance so that we can start exploring the potential to inhibit this molecule with novel therapeutics. At the same time we discovered a novel phenomenon of mutant p53 tumour cells eating other tumour cells. This research will give us an answer whether this eating phenomenon gives mutant p53 a growth benefit and could therefore form the basis of a novel research line.	
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 advance our understanding of the mechanisms underlying mutant p53 function in chemoresistance and tumourigenesis, which could form the basis for further research into novel cancer therapeutics.	
What species and approximate numbers of animals do you expect to use over what period of time?	For the experiments proposed we will maximally use 300 mice in the next two years. Based on the results of these experiments more experiments might be set up in the future using genetically modified mouse models.	

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 be injected with human cancer cells that will start to grow and form tumours just under the skin so we can easily monitor them. Some mice will be treated with cisplatin or doxorubicin at tolerable doses, which have been shown in previous studies to kill the tumours unless they have acquired resistance to these drugs. Adverse effects of cisplatin or doxorubicin at these doses is not to be expected. Once the tumours reach a size that is not yet harmful for the mice, mice will be humanely euthanized and tumour material collected for studies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To further cancer research it is important to take into account the complex physiological circumstances surrounding the tumour, which cannot be replicated in a tissue culture situation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use data from previous studies to assess the number of mice required to obtain statistically significant differences in the conditions we are interested.
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.	Instead of using genetically engineered mice that develop tumours in due course over several weeks or months, we will confine our studies to immune compromised mice. Immune compromised mice are perfect recipients to study the growth of tumour cells as they lack the capacity to reject the xenograft and develop tumours at a quick rate and at a site on the skin that researchers can easily see. This means that mice will only be exposed to a tumour for a short time under very controlled circumstances. Similar mice have been used by others and have successfully revealed mechanistical details regarding chemotherapy and tumour growth. Tumour growth can be monitored by eye reducing the chance of mice getting suddenly ill. Nevertheless, mice will be weighted and monitored regularly to ensure their welfare and will be culled humanely if suffering any more than mild adverse effects.

Project 38	**Combining immunotherapy with current cancer treatments**	
Key Words (max. 5 words)	Cancer, oncolytic virus, magnetic particles, MRI, macrophages	
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 objective of this Project Licence is to determine the effects of various conventional cancer treatments (like chemotherapy and radiotherapy) on the phenotype of immune cells that infiltrate the tumour following such therapy. It is thought these immune cells acquire properties to support tumour regrowth. However, the mechanisms underlying this are still unknown. We will develop new therapies aimed at re-educating immune cells to have anti tumour properties and these will be delivered to tumour using a magnetic guidance strategy.	
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 being made in the treatment of cancer in the last few decades, the prognosis for patients with the most common forms of solid cancers is still poor. New treatments are urgently required. Here we want to understand how current cancer therapies influence cells of the immune system to support the regrowth of tumours. This will help us decide which cells make suitable cell therapies. We then want to use these cells to deliver novel immunotherapies such as cancer killing viruses to tumours and combine this new approach with conventional therapies to completely eradicate the tumour and activate anti-tumour immune responses. This research is of a very translational nature and will yield results of direct practical value for translation into the clinical treatment of cancer.	

What species and approximate numbers of animals do you expect to use over what period of time?	All the animals used in this licence will be mice. Over the 5-year duration of this licence, it is estimated that approximately 1300 mice 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 level of severity? What will happen to the animals at the end?	The likely expected adverse effects resulting from this study are large tumour growth or ulceration of tumours. The severity limit used in this project should therefore not exceed moderate. Any animal showing large tumours (15 mm diameter), ulceration that has not healed or other signs of distress or sustained weight loss during the course of an experiment will be humanely killed.
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To achieve our objectives, we need to use a model system that incorporates an intact blood vessel network, with all the machinery necessary to control its function. This requires the use of animals, specifically rodents. We intend to use mouse tumour models that can copy aspects of the human disease, whilst keeping any distress, discomfort or pain felt by the animals to a minimum.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will always use in vitro models to assess the efficacy of our therapies before using animals. Only those shown to work in vitro will be investigated in our animal cancer models. Statistical tests will also be used based on our previous studies to calculate animal numbers required for our research.
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 the animals used in this licence will be mice. These species have been chosen primarily because of the availability of a variety of tumour cell types that can be successfully implanted into them for the growth of solid tumours and myelomas. Animals will be kept in a filtered air environment to avoid infection and will be cared for by highly trained and competent staff. Husbandry and care will be based on the best practice. All animals that have undergone a procedure will be monitored daily and veterinary advice will be sought if there are any unexpected health problems. At the end of the experiments animal will be culled and tissues and organs removed for investigation.

<table>
<tr><td>Project 39</td><td colspan="3">Validation of Tumour Vascular Targets and Associated Therapies</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Angiogenesis, cancer, vascular targets</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 Years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td colspan="3"><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></td><td>No</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 colspan="3">We aim to investigate targets in the blood vessels of cancers so that we can develop novel treatments. We are focussing on cancer for which there is currently an unmet need including pancreatic, liver and lung cancers.

We aim to investigate the importance of these targets for blood vessel development, tumour growth and dissemination.</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 colspan="3">The project aims to develop data to support clinical trials of anti-cancer therapies targeting tumour blood-vessels.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td colspan="3">3000 mice</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse</td><td colspan="3">The expected severity level is moderate.</td></tr>
</table>

effects and the likely/expected level of severity? What will happen to the animals at the end?	Potential adverse effects: General anaesthesia, which can cause a loss of the bodies heat regulation. The animal's body temperature will be maintained in an appropriate thermostatically controlled incubator after prolonged anaesthesia. Treatment with experimental therapeutics. These may have various side effects. The type of therapeutics proposed for use in the project generally have few side effects, however we will use previous experience and data in the scientific literature to inform our use of these therapeutics. Implantation of tumour cells under the skinor into the mammary fat pad. Both the tumour implantation methods and tumour itself can cause adverse effects such as significant weight loss, distress or pain. Appropriate analgesia will be provided after surgery and otherwise when necessary to reduce pain. The animals will be closely monitored after tumour implantation and killed if any adverse effects are observed which exceed moderate severity. One of the models proposed involves the potential metastatic spread of the tumour, the health of the mice will be very closely monitored to ensure that secondary tumours do not exceed moderate severity. Real time imaging of the living animal and tumour by bioluminescent imaging techniques. This approach involves short to medium term periods of anaesthesia and injection with imaging substrates and contrast agents. These agents are not reported to cause adverse effects. Mice will be killed by a schedule 1 procedure.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Blood vessel development and its promotion of tumour growth and spread are processes that occur in cancerous tumours. At present, we are unable to replicate these processes without using animal models. There are, however, ways to investigate certain properties of vessel development in non-animal experiments. We will use these experiments to their full potential to investigate the role our targets play in blood

	vessel development before moving into animal experiments. We will continue to seek and develop alternatives to animal experimentation throughout the course of this work.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Statistical significance of data and numbers of animals to be used. It is our past experience that data from pilot experiments using five mice per group permits the use of power calculations (MedCalc) to predict the minimum number of mice needed for the study in order to obtain statistically meaningful data. This can vary from the initial five mice in the pilot study, for example, in an angiogenesis sponge assay, to as many as 17 mice per group in tumour growth studies. We will adopt this pilot approach using power calculations throughout the proposed study. An expert statistician will provide advice, although the applicant also has 20 years experience in the application of statistics to in vivo data. Emerging non-invasive imaging techniques (bioluminescence and ultrasound) will be used in conjunction with mouse tumour modelling to generate critical data on tumour vascularity and viability throughout its development. These imaging techniques allow this data to be generated *in vivo* without culling mice at each stage, greatly reducing the number of mice 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.	**Choice of species** As well as the various in vitro models of aspects of vessel formation, for some genes, vessel development in zebrafish embryos is useful in determining the function of genes. Where possible we use this model to give additional insight into the biology of our target genes. Previous research by many groups has documented that the mouse is the lowest organism with sufficiently similar pattern of vessel development to man to allow meaningful evaluation of vessel disrupting approaches to cancer treatment. **Choice of models and methods:**

	Normal blood vessel development experiments: The rodent subcutaneous sponge is considered to be the least invasive methods for the investigation of normal blood vessel development. *Orthotopic tumour models:* It is becoming increasingly clear that the mechanisms behind tumour blood vessel development are specific to each tumour type and location. It is therefore critical to investigate tumour blood vessel development in the appropriate host organ for the cancer type being investigated. Only then can meaningful data be collected that can be translated to clinical trials. *Tumour imaging via bioluminescence and ultrasound:* Tumour imaging via bioluminescence and ultrasound are the least invasive method of *in vivo* tumour imaging available to us for longitudinal studies, requiring only short or medium periods of anaesthesia. Each method has its advantages over the other. Bioluminescence is very effective for rapid screening for tumour growth and invasion. Ultrasound imaging provides more detailed information about the tumour viability and vascularisation. Each will be used appropriately to minimise impact on individual mice whilst still generating meaningful longitudinal data. **Minimisation of animal suffering:** The period of tumour burden will be the minimum required to obtain statistically meaningful data as predicted from power calculations based on the pilot study and in accord with guidelines concerning maximum tumour size. All studies will be carried out in accordance with NCRI guidelines for the welfare and use of animals in cancer research (BJC 2010 102 1555-1577).

Project 40	**Models advancing knowledge and treatment of paediatric brain cancer**	
Key Words (max. 5 words)	Paediatric, brain, cancer, treatment, biology	
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)	Our main objective is to understand the development and find new treatments for three types of paediatric brain tumours. Ependymoma, medulloblastoma, and choroid plexus carcinoma (CPC) are the most common solid cancers to affect children. Each presents a unique set of clinical challenges, and all require new treatments. With few exceptions, childhood brain tumours remain one of the biggest killers from disease in children and require aggressive surgery, radiation and chemotherapy that have changed little in several decades. Radiation is especially damaging to the developing brain and results in devastating long-term cognitive side effects for survivors. Fewer than 70% of all patients are cured following initial therapy and once these tumours recur they have a dismal prognosis. Importantly, ependymoma and CPC are relatively insensitive to chemotherapy and there is therefore a great need for effective new treatment strategies. By investigating the development of these detrimental diseases we will advance the understanding of the underlying biology and by exploring new and innovative treatment strategies we will hopefully be able translate new treatments into	

	the clinic.
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 paediatric brain tumours. The benefits of this project are numerous and include, but are not restricted to: (i) advancing the knowledge of three paediatric brain tumours (ii) Provide brand new and repurposed drugs for clinical testing.
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 11.000 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, limited normal behaviour, loss of movement on one side associated with tumour growth in one hemisphere of the brain. Animals in distress will be humanely killed. 2 protocols are mild, 11 protocols are moderate.
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. Regulatory and research bodies require preclinical assessment of potential therapies in animals models prior to their translation to the clinic. Therefore, our translation of optimal new therapies to the children's cancer clinic requires the animal studies proposed here. Nonetheless we will continue to use in vitro drug sensitivity studies, including radiation/chemotherapy combination studies in vitro to optimise the selection of agents and thereby minimise the use of animal models in exploratory studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our use of in vitro methods limits the number of animals required for the in vivo investigation stage. Furthermore, each of our in vivo mouse model experiments has a careful statistical design that is aimed at minimising the use of animals while ensuring robust and meaningful statistical end points. These animal numbers are selected in collaboration with our statistical colleagues and our extensive

	experience with brain tumour mouse models. In addition we have optimised the use of material from each mouse, often harvesting fresh cells for culture, frozen tumour for RNA and DNA studies and fixed material for histology from the same animal.
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 only species employed in our protocols. They are the least sentient species that best fit our criteria for the following reasons: Their lifespan (approx. 2 years) allows for "development to humane endpoint" studies; the scientific community has a range of techniques to manipulate the mouse genome, allowing us access to many transgenic/knock-in/knock-out mice with which to answer specific key questions; mouse gestation is less than three weeks, and embryogenesis in this species is extremely well documented in the literature, allowing us to look at the effect of genes on normal brain development. Non-animal models cannot recapitulate the complex context existing in developing tissues in which cancers actually form and are treated. The advancement of knowledge and development of concepts to improve human and animal health and well-being requires the use of living animals. Exhaustive literature searches in brain tumourigenesis show that our tumour systems are the most accurate models for the study of these diseases.

Project 41	**Evaluation of novel drug formulations in pancreatic cancer**	
Key Words (max. 5 words)	Cancer, drugs, nanotechnology, imaging, targeting	
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)	Pancreatic cancer is the fourth main cancer in the western world. Pancreatic resection is currently the only treatment known for this cancer with only 5- 34% of patients surviving 5 years after treatment. Currently the only chemotherapy available clinically is gemcitabine, however, this only proves effective in 23.8% of patients. Therefore there is a huge clinical need to explore alternative 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)?	We are conducting experiments in the laboratory to identify new anti-cancer formulations. We hope that these can one day be used to treat patients. It is relatively easy to kill cancer cells in the laboratory, but harder to do it in a patient because the drug may possess poor physical properties which mean it cannot be administered or upon administration it may make the patient ill. Initial studies in animals are required to assess the relative use and safety of such formulations before we can give then directly to humans. To justify doing this, we have to have a reasonable expectation that the drugs will work in patients. We can only do this by testing the drug in animals.	

What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 120 mice per year 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?	Animals will have small volumes of cancer cells injected to allow a tumour to grow inside them. This may cause modest discomfort. The size of the tumour will be carefully monitored and animals will be humanely killed before it becomes large enough to cause distress. Drugs may be administered to the animals by injection. The process of administration of the drug is only expected to cause very mild discomfort to the animals. The adverse effects of the formulations themselves depend on the type of formulation we test and which drug they incorporate. Sometimes we are testing drugs that have been used before and we know what adverse effects to expect. However, many of the drugs are new and although they are designed to specifically target the cancer cells, they may have unexpected effects. This makes it hard to know what adverse effects might occur. To minimise the adverse effects on the animals, we will first test the drug on a small number of animals to identify a dose of drug that has only moderate adverse effects. All further experiments will use lower doses, so we anticipate that the majority of animals will only experience mild severity events. In some studies, we may take blood samples (again with a narrow needle) and only small volumes of blood will be collected as infrequently as possible. In some studies imaging of the animals will be
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	carried out, the animals will be injected IP with appropriate dyes, anaesthetised and placed inside an imaging cabinet. In some studies we will also use laser irradiation to the localised tumour site in order to initiate drug release from formulation. In some cases this may involve minimally invasive surgery dependent on the location of tumour relative to the skin. In the cases where minimally invasive surgery is required

	anaesthesia and analgesia will be administered. At the end of the experiment, the animals will be humanely killed.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We wish to find new drug formulations to treat pancreatic cancer. We are testing formulations which specifically target cancer cells incorporating drugs that have been used clinically as well as drugs that are completely new. We need to gather sufficient information to convince the medical and pharmaceutical world whether our formulations are likely to work in patients — it is not reasonable to give a patient who is already quite ill a new drug unless there is a reasonable likelihood that the drug will do more good than harm. To gather this information, we need to test the drugs using a method as close to real patients as possible. Although we can (and will) test the drugs in the laboratory, these types of studies are not sufficiently complex to model what happens in real patients. Animals are not a perfect model, but they are the closest model we have. Using computer simulations can help, but simulations are limited to testing things we already know about. Our formulations are completely new, or they are established drugs being used in a different way, so we don't know what will happen. We can't make a computer model of something we don't know about.
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 design our experiments to minimise the numbers of animals used. Firstly, all the formulations which are tested in animals will be tested in several different types of experiment in the laboratory to confirm they have the desired anti-cancer activity. We will also try to mimic in the laboratory the conditions in the body. If the drugs do not work in these studies, we will not test them animals. This 'triage" process will minimise unnecessary testing.