Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 1

Projects with a primary purpose of: Basic Research – Oncology

Project Titles and keywords

1. **Renal cell carcinoma pathogenesis**
    - Renal cancer, genetics, carcinogenesis

2. **Centrosomes in development and cancer**
    - Centrosome, chromosome heritance, cilia disease, microcephaly, cancer

3. **Preclinical models of brain tumours**
    - Brain tumours, brain metastases, glioma

4. **Production and supply of Biological Products**
    - Tissues, Monoclonal / Polyclonal Antibodies, Macrophages

5. **Role of cell cycle regulators in gene regulation**
    - Cell division, gene regulation, cells

6. **Cancer and the micro-environment**
    - Cancer, leukaemia, myeloma, micro-environment

7. **Epithelial stem cell plasticity; relevance to cancer**
    - Epithelium, plasticity, stem cells, progenitor cells, cancer

8. **Investigating ubiquitin ligases in disease.**
    - Cancer, neurodegenerative disease, protein degradation

9. **Understanding mechanisms that regulate tumourigenesis and metastasis**
    - Mouse, cancer, metastasis, immune system

10. **Cell motility and tumour progression**
    - Cancer, metastasis, embryonic development

11. **Studying the host response to tumour and metestasis**
    - Cancer – host interaction

12. **Immune signalling in cancer and infection**
    Immune system, signalling, infection, cancer

13. **Analysis of skin carcinogenesis in transgenic mice**
    - Carcinogenesis, Skin, Transgenic, Mechanism

14. **Image Studies of Cancer**
    - Tumour, Imaging, Therapy

15. **Metabolism as a novel target for cancer therapy**
    - Cancer, metabolism

16. **Targeting the mechanistic drivers of lung tumour progression**
    - Tumour progression, therapy, lung cancer

Project 1	**Renal cell carcinoma pathogenesis**	
Key Words (max. 5 words)	Renal cancer, genetics, carcinogenesis	
Expected duration of the project (yrs)	1 year	
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)	Renal cell carcinoma is the most common type of kidney cancer affecting 8,000 people and causing 4,000 cancer related deaths each year in the UK. Despite recent advances in our understanding of the underlying molecular biology and the development of novel therapeutic agents it remains an incurable disease once spread outside the kidney. The study of renal cancer pathogenesis and the development of more effective treatments have been hampered by the limited availability of appropriate genetically-defined animal models of the disease. The development of reproducible and accurate renal cancer mouse models will allow in depth investigations of the underlying tumour biology and the discovery of new methodologies for the detection, management and treatment of human cancer.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The primary potential benefit relates to new knowledge about the initiation and progression of renal cancer. We will aim to publish the findings in academic journals and this information is likely to be of interest to pre-clinical scientists interested in tumour biology. The secondary potential benefit relates to the value of the results to clinicians and to the possibility that new therapeutic targets may be identified, for which new pharmaceutical products	

	could be developed. Thirdly, any developed mouse models of renal cancer will be made freely available to academic collaborators and will represent an invaluable resource for the early evaluation of novel methods for the detection and therapy of renal cancer.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 600 mice over a one 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?	Mice used for breeding and maintenance of colonies are not expected to develop any adverse effects (mild severity). The genetically engineered mice used during this study for the development of renal tumours might experience abdominal distention, haematuria, weight loss and reduced activity (moderate severity). We expect these to occur in less than 10% of experimental mice. Following completion of experimental procedures mice will be killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The elucidation of the genetic events and mechanisms critical for the development of cancer requires investigation within model systems that replicate as close as possible the human disease (cell and tissue of origin, surrounding environment, immune system etc.). These parameters cannot be replicated in culture systems. The laboratory mouse represents one of the best available model systems for cancer owing to various factors including its extensive biological similarities to humans, and an entirely sequenced genome. Furthermore, genetic modification of the mouse genome can be easily and efficiently achieved. An important aim of this project is to generate improved mutant mouse lines that are prone to the development of renal cancer. Such mouse models will be useful to determine the significance of various genes in the development of the human disease. In addition, a robust and reliable mouse model of renal cancer will be a useful system for the pre-clinical evaluation of diagnostic and therapeutic approaches. This model will be a valuable addition to the currently used systems (cell cultures, transplantation mouse models) that have proved of limited clinical prognostic value.

2. Reduction Explain how you will assure the use of minimum numbers of animals	We will aim to minimise the numbers of animals used by: (1) Investigating candidate genes in culture systems prior to generating mouse models (2) Reduce the amount of breeding required to produce experimental and control animals (3) Determining the approximate lifespan of the various renal cancer models in small pilot studies and collaborating with biostatisticians at our institute to determine the minimum number of mice that are required for any studies in order to reach conclusive results with suitable certainty (4) Creating a tissue repository from generated mice to use in future experiments and share with other researchers (5) Ensuring that none of our investigations duplicate work already performed
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 a well-studied experimental species whose genome can be easily and efficiently modified allowing the investigation of complex genetic diseases like cancer. The models used in our studies will allow us to control and direct genetic changes to the stage of development and tissue relevant to the purpose of our studies (i.e. the kidneys of adult animals) thereby limiting the effects of modified genes to other organs/systems. We will only use well established reagents and protocols and where novel methods need to be employed, potential harms will first be carefully characterised in small pilot studies. We will aim to detect the development of kidney lesions as early as possible and therefore limit their effects on the health of the animals by performing regular abdominal palpations and ultrasound imaging. The staff of our animal facility has extensive experience in animal husbandry, welfare and disease and we will take advantage of their expertise to pick up signs of suffering early so that it can be minimised/alleviated.

<table>
<tr><td>Project 2</td><td colspan="2">Centrosomes in development and cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">Centrosome, chromosome heritance, cilia disease, microcephaly, cancer</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2">5</td></tr>
<tr><td rowspan="9">Purpose of the project as in ASPA section 5C(3)

(Mark all boxes that apply)</td><td>X</td><td>Basic research</td></tr>
<tr><td></td><td>Translational and applied research</td></tr>
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<tr><td>X</td><td>Maintenance of colonies of genetically altered animals</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="2">Centrosomes are bodies that organise the dynamic microtubule skeleton of cells to control cell shape and the inheritance of chromosomes at cell division. At the core of most centrosomes are centrioles, organelles that also have another role as the building blocks for both motile and immotile cilia, cellular projections involved in signalling or motion.

- Some centrosomes don’t have centrioles – such as those that organise the meiotic spindle in the mammalian egg cell. We plan to understand the molecular basis for how such centrosomes function and how the egg cell spindle is built.

-We want to learn how centrioles are lost from the progenitors of egg cells and how they are first put together in the developing embryo.

-We intend to understand how centrosomes, with or without centrioles, can respond to orienting cues within a cell to direct the plane of division.

-We aim to understand how centrosomes contribute to organising skin, brain and other tissues by regulating the organisation of the cell and its division orientation.</td></tr>
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	-Because elevated numbers of centrosomes are found in cancer cells we also plan to investigate the consequences of experimentally elevating centrosome number and determine how this affects tumour formation.
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)?	1. The research will help us understand why older mothers have more babies that are affected by abnormal inheritance of chromosomes. 2. We will gain insight into diseases resulting from abnormal cilia for example Barddet - Biedl and Merckel-Gruber syndromes, Polydactly, Cystic kidneys, Oral-Facial- Digital syndrome, Nephronophtisis, Retinitis pigmentosa, Situs Inversus. 3. Knowledge of the machinery that organises microtubules in dividing and quiescent cells in normal cells will help us understand how this goes awry in cancer cells. 4. Discovering how the cell division machinery responds to cellular cues to direct the orientation of cell division will help us understand how bodily tissues are built. We will find out how this goes wrong in the microcephalies (inherited diseases resulting in small brain), diseases in which the brain fails to develop properly. 5. Understanding how cells maintain just two centrosomes and how this mechanism is perturbed in cancer cells that develop multiple centrosomes will give insight into the roles of increased centrosomes in development of cancer.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use an average of 20 female mice/week as source for embryos during the 5 Years of the project. We will also estimate to have an average of 44 cages/week of transgenic mice during the 5 years of the project. Therefore, we will need 5500 females for super ovulations. We also predict that we will use 23500 mice for generation and maintenance of the transgenic and knockout lines required for this study. This will amount to 41,040 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	The greater part of this experimentation is of minimal and moderate severity. Post-surgical complications are unlikely and animals will be given analgesics routinely and monitored closely. Animals that develop tumours will be closely monitored and killed if the

happen to the animals at the end?	tumours interfere with a vital process or they ulcerate or the animals show any other clinical signs. All animals will be killed 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	This study aims to understand the roles of centrosomes specifically in mice because rodents are the only mammals that manage the early stages of development without centrosomes. Thus not only will mice allow us to study cell division in the egg cell but also in the embryo that develops from it. Thus mice are an absolute requirement as experimental material. Once we identify the molecular processes used for cell division in mouse eggs and embryos, we will determine if these can be recapitulated in cultured cells or frog egg extracts. Studies of roles of centrosomes in developing skin and brain also necessitate the mouse model but it may in some cases be possible to substitute with stem cells differentiating in culture. In a similar vein, we will explore the extent to which stem cell models can be used to substitute for mice in studying the relationship between tumour development and centrosome number.
2. Reduction Explain how you will assure the use of minimum numbers of animals	For all experiments we first perform pilot experiments using a small number of embryos to refine the experimental procedure and design. We then expand the experiment to use the minimum number of animals required for statistically significant information. The utilization of mouse lines that express Fluorescent Protein-tagged markers greatly facilitates our observations of spatial events in embryo and means that we can use fewer embryos to obtain significant results. We also routinely use superovulation to increase the number of embryos that can be recovered from one mouse, and thus reducing number of animals required for an 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	We choose the mouse because of the variety of routes used by its cells for spindle formation in cell division and its well characterised development. Mice are also the only species on which, for technical reasons, the genetic manipulations which are essential for this work are capable of being carried out. Work carried out on mouse eggs and embryos entails minimal suffering of adult mice. To study development of the embryonic brain, we have a need

(harms) to the animals.	to electroporate foetuses in utero. The necessary surgery will be carried out with appropriate anæsthetics and analgesics to prevent or relieve any pain and suffering. If over-expression of centriole proteins contributes to tumour development we will killed such mice at the earliest possible stage to avoid suffering.

Project 3	**Preclinical models of brain tumours**
Key Words	Brain tumours, brain metastases, glioma
Expected duration of the project	5 year(s) 0 months

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

Purpose	
Yes	(a) basic research;

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

Brain tumours develop in up to 35% of all cancer patients and they are associated with short survival times of less than 1.5 years from diagnosis. Effective therapies are lacking. Our goal is to develop improved therapies for brain tumours at the pre-clinical level by addressing two of the major obstacles that hinder effective treatment:

(i) Blood vessels in the brain, which are much tighter than in other tissues, hinder the passage of systemically administered drugs from the blood into the brain tumours. Approaches for improved delivery of drugs into the brain are therefore expected to improve therapeutic effects.

(ii) Brain tumours have different characteristics than tumours in other organs and they are often resistant to standard therapies. Therefore, identification and exploitation of molecular targets specific for brain tumours are required.

In line with these unmet needs, our objectives are:

(i) to use a subpopulation of white blood cells, which can efficiently penetrate blood vessels in the brain, as Trojan horses for the delivery of drugs.

(ii) to identify molecular targets specific to brain tumours and perform their validation at the pre-clinical level, thereby enhancing the clinical translation of our findings.

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 findings are expected to inform the development of improved clinical therapies for brain tumours, thereby benefiting cancer patients. In addition, our findings will enhance the understanding of basic biology, thereby advancing the brain tumour research field.

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

We expect to use no more than 3600 mice within 5 years.

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

The vast majority of mice undergoing surgery show mild or no symptoms after they awake from anaesthesia. A small proportion of mice (~0.3%) may experience stroke after the surgery and these mice are immediately culled humanely. Intracranial tumour growth leads to specific symptoms once the tumours become larger; at the experimental endpoint, the majority of mice experience mild symptoms characterized by slight under-grooming. A small proportion of mice is expected to display moderate symptoms including strong under-grooming, reduced mobility, hunched posture and potentially isolation. Only a minor percentage of mice (~0.3%) may display severe symptoms characterized by lethargy or disorientation.

Application of the 3Rs

Replacement

The development of approaches for improved delivery of drugs to brain tumours requires a model in which the white blood cells travel from the bone marrow to the brain via blood vessels. This complex process requires a whole organism and can therefore only be recapitulated in animal models.

Cancer cells growing within their natural environment (in the brain) strongly differ from cancer cells growing in cell culture. Sole analysis of cells grown in cell culture is therefore unlikely to identify good therapeutic targets. Brain is a very complex organ that can at the present not be recapitulated ex vivo, and therefore these studies require use of animal models.

Reduction

The number of required animals is significantly reduced by including ex vivo studies, by using bioluminescence imaging that allows longitudinal study of tumour growth without necessity for multiple terminal end points, and by adequate choice of animal models for brain tumours. Moreover, as many parameters as possible are analysed within one experiment, thus maximizing the outcome per experiment. We also use statistical approaches to calculate the minimal number of animals that will allow us to obtain significant results.

Refinement

Mouse model is an established host model for studies on cancer progression, including brain cancer, and therefore well characterized. Sufficient literature supports the correlation of cancer biology and biology of brain disorders between mouse and human. To model brain tumours, cancer cells are implanted directly into the brain or administered into blood circulation, from where they subsequently enter the brain tissue. All surgery procedures are performed under general anaesthesia and pain killers are administered to minimize any pain potentially resulting from the surgery. Antibiotics are administered to prevent potential infections. Improvement of surgery procedures is an important part of our refinement efforts. Over the years, we

optimized surgery techniques, which resulted in significantly reduced percentage of mice experiencing stroke. Animals are closely monitored - including throughout the night whenever required - to warrant their wellbeing at all times and to ensure that experiments are terminated prior to occurrence of substantial symptoms.

Project 4	**Production and supply of biological products**	
Key Words (max. 5 words)	Tissues, Monoclonal / Polyclonal Antibodies, Macrophages	
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 production and supply of biological products including antibodies and 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)?	The tissues and antibodies produced from the animals used in this licence will enable a wide range of *in vitro* or *ex vivo* studies to be undertaken. These include development of potential clinical applications relating to immunotherapy in cancer treatment. Other benefits are related to the development of new diagnostic tests for infectious diseases in animals and *in vitro* studies increasing the understanding of the mechanisms of ischaemic injury.	
What species and approximate numbers of animals do you expect to use over what period of time?	Mice (1,250) Rats (750) Rabbits (41)	

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?	A proportion of animals will be used for collection of blood and tissues while under deep surgical anaesthesia in a non-recovery protocol. This means that for these animals adverse effects are limited to the gentle induction of anaesthesia and the severity is classed as 'Non Recovery. Animals that remain conscious during blood sampling or are immunised to produce antibodies and other immunologically related cells and tissues will experience the skilled insertion of a hypodermic needle or the minor puncture of a superficial blood vessel. Transient inflammation or irritation may be experienced around the injection site. However significant adverse effects are not expected to occur and the level of severity is classed as Mild. At the end of the protocols the animals will be either humanely killed for the collection of tissue and cells or undergo deep surgical anaesthesia in a non–recovery process to obtain maximum amounts of blood to containing the valuable antibodies resulting from the immunisation schedule.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Research protocols requiring a range of living tissue and/or blood/blood products need tissue of a known biological quality and with an ability to transfer live tissue from the donor into the *in vitro* or *ex vivo* experimental apparatus while maintaining the viability of the tissue. Antibodies are produced by a living immune system involving activation of specific cells in response to antigens e.g. infective micro-organisms or in a laboratory situation specific molecules e.g. proteins. This means that laboratory animals of excellent health status and known genetic background are required to produce the highest quality of antibodies for research.
2. Reduction Explain how you will assure	In the case of tissue collection typically between one to three animals per study. Furthermore, this project will lead to the efficient use of animals by utilising any

the use of minimum numbers of animals	potentially surplus stock animals for the provision of tissues including blood as appropriate. For monoclonal antibody production, extensive experience has informed research groups that, to ensure a good humoral response is obtained in at least one mouse, a minimum of 3 mice per group are required. Smaller groups may lead to waste of valuable antigen, delay in obtaining valuable antibodies if no response is obtained, and requiring repetition with the use of more animals. For polyclonal antibody production, where experience indicated a particularly good immune response can be obtained from the antigen then 2 animals per group may be used. This is particularly the case for the production of polyclonal antibodies in rabbits because it is possible to harvest larger volumes of antibody containing serum from fewer animals. In addition the recognised excellent immunological response of rabbits to a wide range of antigens means that there is an increased likelihood of a successful outcome from the protocol. The less specific response in mice means that typically 3 mice will be immunised for polyclonal antibody production when only small quantities of antibodies are required or only small quantities of antigen are available to stimulate the immune response.
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, rats, and rabbits are all species required to supply tissues and blood products for *in vitro* or *ex vivo* tissue experiments. These species are used extensively to study biological responses relevant to man and other animals. These species also produce antibodies with properties that allow the development of diagnostic tests and novel therapies in man and other animals. The animal species to be chosen for antibody production is determined by the type of antibody required, the volume of serum required, the nature of the antigen and the likelihood of an immune response by the species. Mice and rats are typically chosen for immunisation to provide the required specific antibody producing spleen cells for monoclonal

	antibody production and can be also used for polyclonal antibody production where only small volumes of serum are required. Rabbits are used for polyclonal antibody production due to the recognised good response to a wide range of antigens and ability to harvest larger volumes of antibody containing serum. The ability to produce 'human' antibodies in mice and rats which have been genetically altered to produce antibodies effectively identical to those produced by the human immune system is of significant importance. These antibodies have real possibilities of improvements in the treatment of Cancer using immuotherapy techniques. Animals will typically be group housed and monitored at least once per day by a trained and competent animal technician. Bedding and environmental enrichment will be provided for all animals to enable them to live normal, good quality lives. Experimental procedures may involve a limited number of injections and/or small blood samples (the latter using local anaesthesia) over a period of several weeks. These will be conducted according to best practice guidelines by trained and competent staff. Procedures will be classed of being of Mild severity and have only a transient impact on the animal. Any concerns regarding the health or welfare of an animal will be discussed with the Named Veterinary Surgeon or the humane killing of the animal. At the end of the procedures animals will be killed using a recognised humane method detailed in Schedule 1.

Project 5	**Role of cell cycle regulators in gene regulation.**	
Key Words (max. 5 words)	Cell division, gene regulation, cells	
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)	For a single fertilised egg to give rise to an adult human, cells must undergo trillions of divisions, each giving rise to two separate daughter cells. At the same time that this is happening, the cells are specialising to give different types of tissues (eg. liver, kidney or brain). Specialisation involves switching on of specific sets of genes for each tissue and silencing of other genes. All of this must happen in conjunction with the very complex and disruptive process of cell division. In fully specialised tissues, most of the cells stop dividing. Failure to arrest cell division at different points in the specialisation process results in cancer. We are studying how expression of genes associated with different stages of the specialisation process is controlled and synchronised with cell division. We aim to understand how this synchronisation affects the decision that cells make to turn into the specific types that are found in different tissues. We are also studying how proteins that are involved in regulating cell division affect gene expression and thereby contribute to the	

	development of 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)?	Cancer is one of the major health burdens in the modern world. Our work is expected to provide insights into why cancer cells divide uncontrollably and how they become more aggressive over time. This information could help with identifying novel targets and strategies for targeting specific cancers. Our studies are also expected to provide novel fundamental information on how stem cells gain the ability to give rise to many different specialised cell types in the body. This may help with the development of strategies for generating tissues for regenerative medicine. A major benefit of our work will be advancement of knowledge in the fields of cancer and stem cell differentiation through publication of findings in peer-reviewed journals.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice: 9000, 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 severity level for the majority (85%) of our experiments is mild. Approximately 15% will be moderate severity. Much of our work is carried out *ex vivo* on cells that have been isolated from genetically modified mice. Most of the mutations are conditional, which means that the mutation is only induced in the isolated cells and there is no effect in the living animal. In some cases, the mutation is induced in the living animal, but only in a specific tissue in the adult. This limits the effect of the mutation on the animal. We also generate a small number of transgenic or genetically modified animals. The severity of the surgical procedures for implanting genetically modified embryos into foster mothers is moderate. Pain and distress from surgery will be minimised by appropriate use of anaesthetics and pain relieving drugs. Animals will be killed by a Schedule 1 method at the end of each experiment. Animals exhibiting any

	unexpected harmful effects will either be killed, or in the case of effects that are of particular scientific interest, advice will be sought from the local Home Office Inspector on how to minimise distress while the phenotype is examined. Any animal displaying signs of ill health will be examined by the in house vet. If the animal fails to respond to treatment or its condition deteriorates, it will be humanely killed by a Schedule 1 method. For all procedures, animal technicians who care for the animals will be briefed thoroughly on the effects of the procedure or any resulting phenotype on the welfare and behaviour of the animals.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Cells isolated from animal tissues provide the only means for studying the cell cycle of normal cells. It is essential that we understand how the cell cycle is regulated in normal cells if we are to gain insights into how these processes are disrupted in cancer. The difficulty of culturing many normal cell types makes it difficult or impossible to introduce subtle genetic modifications into these cells in isolated cultures. This makes it necessary to introduce the mutation into live animals, which can then be used to isolate the modified cells. The interactions of the mechanisms that regulate gene expression and the cell cycle during mammalian development depend on complex contacts and changes that occur in the developing embryo. These can only be studied using live animals. Wherever possible, we use non-animal alternatives. For example we carry out many of our experiments on embryonic stem cells, which are grown in culture, genetically modified using a variety of techniques and differentiated into different cell types in vitro. The extensive use that we make of ex vivo cell culture of cells isolated from animal tissues also constitutes replacement as it replaces procedures that would otherwise be carried out on live animals.
2. Reduction	For the ex vivo analysis of isolated cells that is used

Explain how you will assure the use of minimum numbers of animals	for most of our studies, methods for isolating cells have been optimised to reduce the number of cells required. A careful assessment of the numbers of animals required for each experiment is carried out before the experiment is started. This includes consideration of the type of statistical analysis that will be carried out and the number of animals that will be required to reach statistical significance. Our use of CRISPR targeting has greatly reduced the number of animals required for introducing genetic modifications into mice by eliminating the need to generate chimaeras from genetically modified embryonic stem cells and the extensive breeding of the chimaeras that was required to establish modified mouse strains.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The mouse is the most tractable mammalian species for studying gene expression and the cell cycle because of its short breeding cycle and because of the amount of information that we have about its embryology and physiology. For the same reasons, it is by far the best mammal to use for genetic manipulation. All of the procedures in this licence are classified as either mild or moderate and are done under local, general or terminal anaesthesia, where appropriate, to minimise stress and suffering of the animals. Where appropriate, pain relief will be provided.

Project 6	**Cancer and the micro-environment**	
Key Words (max. 5 words)	Cancer, leukaemia, myeloma, micro-environment	
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)	These studies will significantly help us to achieve our labs overarching aim to develop novel therapeutic strategies to treat human blood cancers through a better understanding of how the tumour interacts with the surrounding non-cancerous cells within the body which support it. The plan is to use these experiments to validate and develop our extensive in-vitro findings on tumour microenvironment interactions between the cancerous and non-cancerous cells and then test the targeting of the discovered processes as a means to treating cancer. Cancer development is a complex, multi-step process that involves interaction of the tumour cells with the local environment and the immune system and cannot currently be recreated by culture methods. Animal models become necessary because the nature of these pathways and drug treatments require the presence of the cancer and its microenvironment in the setting of a whole animal. Animal studies will only be undertaken after full in- vitro investigation and experiments designed to give answers in confidence	

	using the least number of mice. The mouse is the most appropriate and a widely used model for blood cancer research. The techniques are well established, with protocols developed and designed over a number of years specifically to minimize suffering, experimental strains are already available and genetic modification techniques are well established.
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)?	Presently treatment outcomes and survival of patients with the blood cancers acute myeloid leukaemia (AML) and myeloma is poor. Despite current therapies approximately 80% of patients diagnosed with AML die of their disease and most within a year of diagnosis. Myeloma is incurable for all patients with an average life expectancy of about 4 years following diagnosis. We have reached the ceiling of what can be achieved with conventional cytotoxic chemotherapy and therefore novel approaches are needed to improve outcomes for patients. Work by our lab and others shows clearly that AML and myeloma are both highly dependent on the non- cancerous cells that surround them for their growth, proliferation and chemotherapy resistance. The experiments described in this application are designed to develop our understanding of these tumour- microenvironment interactions and take this knowledge towards clinical use in patients. The ultimate aim of this work is to inform the clinical trials of new treatment strategies in patients affected by these blood cancers.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice Approximately 9000 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?	These studies are fundamentally designed to look at the causes of and then to cause and explore the treatment of blood cancer. Therefore most of the animals are expected to develop cancer within this project and many will receive treatment. This is expected to place many animals in the moderate category of severity. Animals which do develop haematopoietic malignancies may experience abdominal distension, weight gain or loss, anaemia,

	laboured respiration, inactivity or inappetence, combined with signs of hunched posture or piloerection. Animals showing these clinical signs will be immediately killed by a schedule I method in accordance with the details set out in this licence. Cancer assessment will be undertaken in accordance with the principles set out in the 'Guidelines for the welfare and use of animals in cancer research: British Journal of Cancer (2010) 102, 1555-1 577.'
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Cancer development is a complex, multi-step process involving interaction of the tumour cells with the local environment and the immune system and cannot currently be recreated by culture methods. Cancer (and its treatment) is not considered in isolation in the clinic and must be considered in the context of the whole patient. Initial testing of the efficacy and any possible side-effects of drugs or any other treatments requires an in vivo model of the disease; however, dose response curves will be determined in culture systems or from published data to provide optimal dosing. We will look to use our data on new candidate genes and molecules involved in AML and myeloma regulation from in vivo culture experiments to inform and develop alternative in-vitro assays for future use.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have an internationally recognized and well-established in-vitro research program from which we will generate as much informative data as we can to limit the necessary in-vivo assays to a minimum. Colony sizes will be carefully managed to ensure that supply matches demand. Whenever possible, surplus mice/tissues will be used for other scientific purposes. Regular reviews will be carried out to ensure that those lines that are no longer or only sporadically required are cryopreserved. Standard operating procedures (SOP's) and data analyses will be continuously optimised to use the fewest number of animals possible. When designing the experiments we will perform statistical analysis to ensure that we use the minimum number of mice per group that will be informative. In order to reduce the number of breeding

	pairs, we will keep some mice as homozygous and/or compound heterozygous for multiple alleles, provided that this does not produce a harmful phenotype. Wherever possible potential therapeutic agents will be pre-screened in vitro to predict the minimum dose that is likely to be effective, thereby reducing the numbers of animals used in in vivo 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.	The mouse is the most appropriate and a widely used model for blood cancer research. The techniques are well-established, experimental strains already available and genetic modification techniques are well-established. We will use and refine established reagents and protocols to induce leukaemialmyeloma and look to develop inducible gene expression or deletion of novel candidate genes. All these measures will be employed where possible to avoid unexpected pain and suffering. For transplantation of blood cells into host animals we will aim to use systems that will minimize the suffering.

Project 7	Epithelial stem cell plasticity; relevance to cancer	
Key Words (max. 5 words)	Epithelium, plasticity, stem cells, progenitor cells, cancer	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		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 way cells behave during development and injury share similar features with tumour cells during cancer formation. Understanding how the behaviour of these cells is regulated in animal models of development, wound healing and tumour formation will help to decipher regulatory processes of potential clinical relevance for the treatment of cancer patients and tissue repair therapies such as burn injuries.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Identifying how the cell behaviour is regulated can help understand how adult stem cells work and, in turn, how cancer cells alter this as disease progresses. Further research in this field is needed to unveil the full complexity of stem cell behaviour. And, in the long term, these studies will determine whether these mechanisms can be medically manipulated in the benefit of patients with skin diseases, including cancer.	
What species and approximate numbers of	This project will use mice, including genetically modified strains. The maximum number of mice that	

animals do you expect to use over what period of time?	could be used over a 5 year period would be 14,740.
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 includes protocols to investigate cell behaviour in wound healing, early tumour formation and development. To do this we will use techniques such as drug administration by for example diet, food or water to genetically modified animals in order to label cells, wounding by biopsying the oesophagus and/or making small cuts in the skin, as well as giving drugs that cause cancers. Sometimes we may replace cells that we remove by modified cells using transplantation techniques, so that we can study how cells behave. Surgical procedures are carried out aseptically under anaesthesia and animals receive analgesics during and after surgery. All animals are checked regularly and if there are any concerns animals are examined and weighed. The majority of the animals (>95%) are not expected to show signs of adverse effects. However, some weight loss with or without other clinical signs such as piloerection, hunched posture may be seen. In these cases, if the signs do not resolve within 24 hours or the animal deteriorates it 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	The project looks at cell behaviour and how this changes during development, healing or cancer over several months. Most techniques working with cells outside living organisms do not fully recapitulate in vivo behaviour over long time points, these approaches are of limited use for the proposed project. Nonetheless, whenever possible, we will implement ex vivo(in tissues outside body) techniques as they are very valuable to screen, validate short term cellular responses, and obtain preliminary data to inform experiments with animals. Importantly, this will replace animal usage in the current project.
2. Reduction Explain how you will assure	By labelling cells using fluorescent markers fewer mice can be used compared to conventional

the use of minimum numbers of animals	approaches as hundreds of clones may be analysed per animal. This represents a significant reduction compared to traditional approaches where an increased number of animals were required to understand changes in cell behaviour. Additional reduction will be achieved by implementing appropriate breeding strategies, adequate experimental design, and the use of ex vivo systems, whenever possible.
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 as these are the only species in which the adequate tools to perform fluorescent genetic labelling are available. All methods proposed in this project use refined techniques that minimise animal stress. These include refined oesophageal biopsy using endoscopic techniques with minimal side effects as opposed to the traditional usage of sharp dental tools. Cell transplantation approaches within the same animal where cells were obtained from will minimise rejection producing more reliable data. Ex vivo studies will be used to plan experiments with animals, reducing number of animals and refining the experimental design.

Project 8	**Investigating ubiquitin ligases in disease.**	
Key Words (max. 5 words)	Cancer, neurodegenerative disease, protein degradation	
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)	Proteins in cells are responsible for controlling how cells function and respond to their surrounding environment within an animal, including in humans. Therefore the abundance of proteins is very tightly controlled, either by regulating how much of a protein is made, or how is it destroyed. We aim to investigate the function of several proteins (called F-box proteins) that are believed to cause disease, by controlling the degradation of multiple other proteins. One particular protein called Fbxo7, when mutated, can cause Parkinson's disease, and it can also contribute to cancer development, so determining how it functions, and what proteins it destroys is very important for human health. We also aim to develop new cancer therapies that can either prevent the function of a different F-box protein that causes cancer, or destroys a protein that is accumulated in cancer because it cannot be degraded effectively.	
What are the potential benefits	The experiments we have planned will help us prove	

likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	whether or not the F-box proteins tested do cause or contribute to diseases such as cancer, and will help us determine how they do this by determining which proteins they are responsible for destroying. This will allow us to decide whether they are likely to be a good avenue to pursue for the development of new therapies targeting these diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	To determine whether F-box proteins cause disease we will use both experiments in the laboratory using cells grown in dishes, as well as mice that have been genetically altered to lack these F-box proteins, either in specific organs or throughout the mouse. We will breed these mice to produce enough offspring for analysis and expect that over 5 years we will need 3550 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 vast majority of mice bred and used throughout this project will develop normally and show no signs of distress or suffering. Some may show abnormal signs such as a smaller size, anaemia, increased chance of cancer development with age, or clinical signs associated with Parkinson's disease, such as a tremor or decreased coordination. These mice will be identified shortly after birth and monitored carefully to make sure they do not show any signs of suffering. We are also developing two novel cancer therapies that will be tested in mice that have tumours to test their anti-cancer effects. These mice will be bred to spontaneously develop tumours, or will be injected under the skin with cancer cells, prior to treatment. The health of these mice will be carefully monitored and mice killed if tumours adversely affect their health. All mice will be kept with companions, if possible, and with sufficient bedding and environmental enrichment, in climate controlled cages with constant supply of food and water. Occasionally we may take a small tissue biopsy for testing but this will cause only transient discomfort and no lasting harm. Additionally mice may undergo non-invasive behavioural testing to examine their normal behaviour, such as their range of movement, sense of smell, psychological state, and balance and coordination. All mice will undergo trial runs so that

	they acclimatise to each test to reduce the stress associated with handling and unusual situations. Therefore these examinations are expected to again cause only transient stress and no lasting harm. All mice will be humanely killed at predetermined time points, the end of the experiment or at any point if they show signs of suffering which cannot be rectified by minimal veterinary intervention.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The function of proteins in cells, and how these cells interact in whole animals, is extremely complex with many counteracting and parallel pathways controlling the same outcome. Therefore we cannot easily use single cell systems in a laboratory to ask how a particular protein functions in multicellular organizations, particularly in complex organs such as the immune system or brain, where multiple different types of cells exist and all interact. Therefore we use mice, as they are the least sentient mammal with similar developmental processes to humans. When at all possible, we will use experiments using cells, both to confirm and expand our knowledge found in mice, and also to develop potential new therapies before testing in animals. We will also use emerging technologies that will allow us to use patient samples (biopsies) instead of animals where possible.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Before any breeding commences we will carefully plan experiments to determine the minimum number of animals needed to produce a significant and reproducible result. We will consult with other experts, such as statisticians, to help with this. If possible, the same animals will undergo behavioural testing at different points throughout their life to greatly reduce the total number of animals needed. We will monitor these mice carefully to ensure that they recover fully between tests, from the stress of being in unexpected situations, and that the cumulative effect does not cause unnecessary harm. We will also perform pilot studies testing new therapies (already tested in isolated cells) in very small groups of normal mice to determine their safety

	before testing their effectiveness in mice with tumours to minimise suffering in these animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We plan only to test the function of F-box proteins with clear clinical relevance (i.e. where analysis will directly impact our understanding of human diseases). We also plan to test the effectiveness of new therapies only in mice likely to respond to treatments, for example, in those with tumours caused by mutations in proteins targeted specifically by the therapies to be tested. All mice used under this license will be regularly monitored for signs of pain and distress, and if any are found, mice will be promptly treated wherever possible (for example with the use of different diets, analgesics, and/or antibiotics), and if suffering cannot be promptly alleviated, mice will be humanely killed. Any mice undergoing behavioural tests, for example to determine their range of movement and coordination, memory, and motivation, will be trained before measurements are taken, to minimise the stress associated with being in unfamiliar environments. If surgery is required, the highest possible standards of care and asepsis will be used, to aid in the quick and painless recovery of animals.

Project Title 9	Understanding mechanisms that regulate tumourigenesis and metastasis
Key Words	Mouse, Cancer, Metastasis, Immune system
Expected duration of the project	5 year(s) 0 months

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

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

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

Almost 1 in 4 people in the UK will develop cancer at some point in their lives. Although great advances have been made in cancer biology with many patients now being cured, it is still a devastating disease that can be hard to treat for some cancer types, especially if the cancer cells have spread to other organs of the body. Critical to improved patient care is a deeper understanding of the biology of cancer, which can pave the way for the development of new therapies.

The specific aspects of cancer we are focussing on are ‘tumourigenesis’ (the initial formation of the tumour) and ‘metastasis’ (the tumour cells ability to grow at a secondary site). Both these events are multi-step processes that depend on the accumulation of mutations within the cells that allow them to become cancerous. Thus knowledge of the key genes that control this processes is critical – genes that when mutated result in a cancerous cell.

However, this is only part of the story, as factors ‘outside’ of the tumour cells, i.e., what is going on in the body, also have a key role to play in both tumourigenesis and metastasis. This can include the normal cells around the tumour cells and critically the immune system. Thus understanding of the way the body can ‘control’ the ability of the tumour cells to grow and spread to other organs provides avenues for potential therapies, as highlighted by the success of “Ipilimumab” – a drug that works

to activate the specific cells of the immune system that are able to kill off cancer cells.

The aims of this work are:

1. To identify genes found in cancer cells that when changed/mutated can affect tumour growth and metastasis.

2. To identify how factors such as the patient's immune system influence tumour growth and metastasis.

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

An understanding of the genes that are altered in cancer cells, and how they work to 'alter' the normal functioning of the cell, is critical if we are to have any hope of identifying ways in which we can 'kill' the cancer cells, i.e., the development of drugs/therapies that are able to target these 'altered' cells and leave normal 'healthy' cells alone. Similarly, if we can find ways in which the body is able to control the growth of tumour cells or prevent their growth at new tissue sites, then this information can be utilised by pharmaceutical companies to develop drugs that target the tumour cells or help the ability of the body to fight them.

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

It is expected that a maximum of 150,000 mice will be used over the course of 5 years, with 50,000 of these being used solely for breeding to generate mice for analysis.

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

In our licence, 60% of the mice we propose to use will experience no more than at most a transient feeling of pain, such as when they are administered substances via an injection into the tail vein or when a blood sample is taken and are considered to be 'mild' in terms of severity. The remaining mice (40%) will undergo more experimental procedures and thus are classified as 'moderate' severity. These mice may carry an altered gene and are monitored to see if they develop a tumour. Alternatively mice can be administered tumour cells and their ability to control the tumour or the spread of the tumour is investigated. We also characterise the immune system of the mice. All mice are monitored daily for any signs of a developing tumour or signs that the animal may be starting to experience abnormal clinical features that suggest it is no longer able to tolerate the presence of the tumour (be it a swollen spleen due to the development of lymphoma or metastatic tumour cell growth in the lungs starting to make breathing laboured). Since animals can also develop tumours internally (i.e., where the tumour growth/mass cannot be directly observed), we use

other signs to indicate the mouse is starting to become unwell, such as coat condition, pale and cold extremities, reduced movement and/or social interaction. At the point when the mouse starts to display these clinical signs, it will be humanely killed and the mouse examined to determine why it was displaying these symptoms, as well as tissue samples collected for further analysis.

Application of the 3Rs

Replacement

Humans and mice share many similarities, both in terms of our basic organs (as we are both mammals) and our genetic make up (there is a very high degree of similarity in the actual genes that both mice and humans share). When mice develop cancer (either due to our alteration/mutation of their genes or due to the administration of tumour cells to them), their tumours are very similar to that seen in humans (in terms of the way the develop and their actual characteristics). Also, using the mouse means we can look at the way the body reacts to the cancer cells and how factors such as the immune system try to control them. This is something that simply cannot be performed by growing cancer cells in a dish in the laboratory.

Thus mice are a very good model for human cancer, and allow us to perform studies that cannot ethically be done using human subjects. Importantly, mouse studies have enabled the development of clinically relevant agents in cancer treatment, such as the development of targeting antibodies that are currently being used to cure patients with advanced melanoma (antibodies that target two proteins on the surface of the immune cells that are able to kill the cancer cells). Indeed, although these are only two examples virtually every compound used in the oncology clinic was developed or validated using mouse model systems and mouse models have also contributed significantly to our fundamental understanding of the mechanisms of cancer.

Reduction

Where possible we shall always import existing mice rather than generating new ones.

In some circumstances, such as when certain mouse lines have been only recently created, less published data will be available and in these instances we propose to perform small pilot experiments to determine the final experimental design.

All mouse lines will be archived so that they may be distributed to other researchers worldwide. This will reduce the number of animals used globally, as fewer animals will be required to re-generate these archived lines.

Data will be generated from the statistically determined minimum number of animals, and wherever possible, experiments will be designed to avoid the known sources of variability that can arise.

Wherever possible, multiple experiments will be performed on the tissues collected from an individual mouse so as to maximise the use of the mouse.

All data generated from our research on the mice will be published in scientific journals available to the whole scientific community, reducing duplication of production resources and phenotyping procedures elsewhere. Wherever possible, the results of experiments that involve large datasets will be made publically available to serve as a resource for other scientists and clinicians

Refinement

We are constantly refining our experimental processes to minimise harm and reduce adverse effects on the mice without affecting the experimental data. For example, when taking blood samples we have refined our protocols such that only a drop of blood is needed to be able to complete our analysis, thus minimising the distress to the animal.

When mice are to be irradiated (given gamma-radiation to wipe out their bone marrow prior to transplant of donor bone marrow), we have instituted a new policy whereby the mice must be weighed 24 hours beforehand and their condition thoroughly observed. This allows us to avoid irradiating mice that may be rather small in body weight and/or may have started scratching (for example) and would be less likely to tolerate the irradiation procedure. We also ensure the mice are placed on antibiotics for 2 weeks after the irradiation, whilst their immune system is compromised, and we also provide mash in dishes on the cage floor for the first week after irradiation to ensure that should they feel slightly weak/tired (as some patients can feel after irradiation therapy), they are still easily able to access food.

Mice are social animals and thus wherever possible we try not to house them on their own. However, in cases where we observe fighting in a particular cage of mice, the aggressive mouse will be removed and solo-housed, so as to prevent further harm to the rest of the cage.

We have highly trained technicians looking after the mice, and the mice are checked every single day to ensure they are healthy. Those that are being observed for the development of tumours are observed twice daily and humanely sacrificed if they are starting to show any signs of discomfort.

The implementation of a sophisticated database system and animal tracking system ensures that data on procedures and welfare assessments can be readily accessed and analysed.

Project Title 10	Cell motility and tumour progression.
Key Words	Cancer, metastasis, embryonic development,
Expected duration of the project	5 year(s) 0 months

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

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

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

The aim of this project is to understand basic regulatory mechanisms that govern cell motility (movement) and cell proliferation (formation of multiple cells) in the whole animal. This project also aims to explore the consequences for cancer formation and metastasis when these regulatory mechanisms are defective. Unfortunately, we don't know enough about these regulatory mechanisms to understand how our body develops and how defective regulation causes disease. Therefore, this knowledge will not only advance science but may provide the basis for the development of new therapies and novel medicines for treating diseases including cancer in the future.

The body is formed of millions of cells, each with their own specific function. The control of cell function and movement is vital to the development and well-being of any organism, and the body has numerous systems involved in these processes. Some cells can react to signalling compounds that are produced and released by other cells. These compounds are often proteins, the production of which is controlled by the genetic material (DNA) found in the nucleus of cells. Cells will respond to these compounds by reorientating their front towards the signal and then they will migrate towards the signal until they reach their target. For example, during development of an embryo, nerve cells will migrate to their correct position and will make connections with other nerve cells, thereby helping to form the brain. In another example, cells of the immune system sense bacteria that have entered the body through a wound. They will then migrate towards them and kill the bacteria, thereby defending the body. However, if cell migration occurs in an uncontrolled fashion this can lead to the development of abnormalities in a developing embryo or

may contribute to the development of disease. For example, in the process of metastasis, cancer cells leave a tumour and spread throughout the body to establish themselves in many sites. The spread of cancer in this way is a major cause of cancer related deaths.

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)?

A greater understanding of the mechanisms that control and influence cell motility and cell proliferation obtained from this project will provide information of great benefit to understanding the processes that are involved in both normal cell function and in examples of altered cell function. This will be important to our understanding of disease processes including developmental abnormalities (e.g. within the developing embryo) and cancer. Therefore, this knowledge will not only advance science but may provide the basis for the development of new therapies and novel medicines for treating diseases including cancer in the future.

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

We expect to use approximately 3700 mice over five years.

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

We will use the animals to perform studies to better understand embryonic development and tumour formation and progression. Animals may show developmental defects or may develop breast tumours and metastatic tumours in the lung. Tumour burden will be limited to the minimum required for a valid scientific outcome. Rarely, tumours may ulcerate. 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. Animals will be humanely killed at the end of each procedure. We have also included several guidelines that regulate when animals should be humanely killed before the end of the procedure when adverse effects getting over a defined threshold.

Application of the 3Rs

Replacement

The migration of cells and the formation of connections between nerve cells are highly dependent on compounds secreted by, and cell surface proteins found on, surrounding cells and tissues throughout the intact animal. Therefore, isolated cells

grown in the laboratory can only be used for the initial preliminary studies and we have to generate genetically altered animals in order to understand the function of a protein within the body.

Furthermore, 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*.

Reduction

We will keep animal numbers low by breeding only the required numbers to maintain the colony and humanely kill a small number of these mice to obtain tissue and cell samples for our studies. We will use the tissue samples to see whether the different tissues of the body have formed and function properly. We will also isolate cells from various parts of the body. To study their motility we will observe the cells with a microscope and film how they respond to proteins (or other compounds) that are added. To minimise the number of mice used for this purpose we will employ a special technique that enables the cells to survive for a long time grown in plastic tissue culture dishes in the laboratory.

For the tumour formation and metastasis assays we use statistical calculations to help determine the lowest number of mice to use that will still give us a trustworthy result.

Refinement

We will use mice for these studies because the technique for the genetic alterations has been developed in this species, and they are the lowest vertebrate animals that are still comparable to humans.

Competent, well-trained staff will be responsible for the care and husbandry of the animals. We will observe the animals very closely in order to monitor any potential adverse effects and to ensure that suitable action to minimize animal suffering is being taken.

Project Title 11	Studying the host response to tumour and metastasis
Key Words	Cancer - host interaction
Expected duration of the project	5 year(s) 0 months

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

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

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

The relationship between tumour cells and their microenvironment has become a vanguard of cancer research. This relationship can be simply expressed by the idea that cancer cells would not be able to start and maintain tumour growth without an active commitment and changes in the surrounding host tissue. This relationship between tumour cells and their associated host tissue persistently characterizes tumour growth, from onset to metastasis and has been recently correlated with resistance to chemotherapy. We aim to study the interaction between cancer and the host to address the the following questions:

1. Identify novel essential cell components and signals within the metastatic and tumourigenic organ environment essential for tumour growth. This will allow us to identify novel target for anti-cancer therapies.

2. Characterize the activity of certain cells normally involved in the protection of our body from infections during cancer. As these cells are reported to act both as pro-tumourigenic and anti-tumourigenic, differentiate what make this switch could allow us to use them against the disease.

3. Explore what kind of inflammatory stress trigger disseminated dormant cancer cell reactivation and relapses in cancer patients that were “clinically cured” more then 5 years before. This understanding will greatly help the management of cured cancer 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)?

The immediate benefit of the project will be the discovery of novel players and mechanism driving cancer onset and progression and in longer period to identify pharmaceutical strategies to interfere with those mechanism and design better anti-cancer treatments.

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

We will use mice only. Based on the work carried out in the past 5 years by my laboratory under our previous licence, the number of mice expected to be used over the 5-year period will be approximately 50000 mice bred 15000 mice used under the various experimental protocols.

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

The studies performed under this project licence will be tumour studies. In addition to the close monitoring of the mice under an experimental protocol, the use of known and as controllable as possible models or protocols to induce the cancer disease will be a strategy to limit the adverse effect. The expected level of severity for all the experiments is either mild or moderate. The majority of the mice will undergo schedule 1 killing at the end of the procedures. On more rare occasion terminal anaesthesia will be required.

Application of the 3Rs

Replacement

In contrast to other tumour studies, here, a tumour is analysed in the context of its local microenvironment as well as the systemic changes induced in the host organism affected by the disease. Therefore this study must mainly be performed *in vivo* as the complexity of these changes and the number of players involved cannot be modelled *in vitro*.

However, targeted *in vitro* assays will be designed accordingly with the gained information used to reduce mouse workload. For instance, in order to assess specific interactions between tumour cells and certain components of the microenvironment or of the immune system. This approach will also have the advantage of identifying the impact of these components on each other in a “clean” system where all the players are known.

Reduction

We employ several strategies to try to limit the number of mice in the study. Firstly, we will always aim to maximise the amount of data we get from each mouse and

when possible, we will use it for the study of both primary tumour and metastasis. Also, we will limit the use of genetic models (that often require many generations breeding) using orthotopic transplants of labelled cells and treating the mice with chemical agents either to block immune-system components or to generate tumours. We also use the minimal amount of mice needed for statistical significance when testing the experimental hypothesis. Finally, by careful monitoring of our transgenic mouse colonies we try to breed as few mice as possible.

Refinement

The scientific question to be address with this project is the understanding of the complex interaction of the cancer disease with the entire host organism, therefore the closest model to the human disease need to be used.

The reasons why mice are the best choice as cancer experimental models, can be summarized as follow:

- the physiology of cancer in mice is consistent with the human disease,

- the need for working with genetic modification (knock out, transgenic models). In mice, many models are available as well as well defined techniques for de novo production,

- they are economic, easy to handle, they produce multiple offspring and they have a very short gestation period as well as a functional survival time.

When working with animal models it is essential to minimise any possible adverse effects of the experimental procedure. We closely monitor the animal’s reaction to specific experimental procedures and pay attention to any sign of sufferance.

Some of the genetic alterations , for instance, mice carrying genetic predisposition to tumour, may show a higher risk of mortality especially after 7-8 months of age. The colony of these mice will be kept as young as possible and monitored closely for the expected potential risks.

When implementing surgical procedures standardized by other laboratories, they are always further refined to the best possible standard. For instance, the size required for the insertion of the scaffold in protocol 15 was reduced to the minimum required. Similarly the position of the incision was changed to avoid contact with the upper metal grid or plastic in the cage.

Project Title 12	Immune signalling in cancer and infection
Key Words	Immune system, Signalling, Infection, Cancer
Expected duration of the project	5 year(s) 0 months

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

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

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

White blood cells of the immune system recognise and target unwanted substances, including viruses and cancer cells. Killer T cells - a type of white blood cell - are crucial for our body's defence against infection and tumours due to their ability to specifically kill infected and tumour cells.

In previous studies we have discovered a protein involved in communication of Killer T cells called Hedgehog (Hh). Hh is helping the Killer T cells to recognise and destroy tumour cells or cells that are infected in cell culture.

In our future studies we want to understand how Hh works in Killer T cells and other immune cells and aim to determine the importance of Hh in immune cells throughout the immune response against cancer and infection. We will also study mechanisms by which we can alter Hh in immune cells in order to increase function in these 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)?

To date very little is known about Hh in immune cells. Our work will not only greatly advance our knowledge about this protein but may also uncover novel therapeutic opportunities for patients suffering from infection and cancer. Importantly, Hh is also active in tumour cells and many cancer patients are thus treated with Hh inhibiting drugs. However, our previous work indicates that this may diminish an immune response against tumours. The work outlined in this project licence will determine

whether activation of Hh in immune cells is a better strategy to treat cancer patients in the future.

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

Over the 5 year course of the licence we expect to use an estimated 19,500 mice.

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

The majority of mice used on this licence will be genetically manipulated mice used for the purpose of breeding and for experiments carried out using tissues from dead mice. Most genetically manipulated mice will be healthy and show no deleterious effects. The mice are maintained in individually ventilated cages in a barrier environment to protect them and ensure any effect of the genetic manipulation on the immune system does not compromise the health of the animal. The majority of the mice on this licence, which undergo a regulated procedure, will only ever experience mild distress – such as a single injection or blood sampling procedure, except in exceptional circumstances. This means that the mice will experience no more than transient discomfort and no lasting harm. In some circumstances, mice will be used for a disease model, either a model of infection or cancer. These mice may experience a higher level of distress, but in these cases the health of mice will be regularly monitored. Any mouse losing weight or exhibiting signs of ill health or pain will be humanely killed. All animals will be killed by an appropriate humane method at the end of the study.

Application of the 3Rs

Replacement

We plan to minimise the number of mice used by carrying out as much preliminary work as possible not using live animals and working in tissue culture. These studies will allow us to optimize and focus our studies on animals but cannot replace them. Immune responses are complex and require a coordinated response from a larger number of different types of white blood cells during infection and tumour challenge.

Our combined approach, using human and animal data, will allow us to explore immune mechanisms that are relevant to human disease, and to translate our results into applications of direct benefit to patients, for example new targeted immunotherapies.

Reduction

We keep the number of mice bred to a minimum, centralising the administration of our mouse strains to ensure coordination of different studies to prevent the duplication of experiments. Experiments are designed using statistical calculations to

ensure results are informative and scientifically valid. We maximise the information we get from an individual animal by measuring the most variables we can from each mouse.

Refinement

The parallels between the human and mouse immune systems are well understood and characterized, which makes the mouse a good species for these studies.

Where possible we perform studies which allow us to examine the immune system using tissue from dead mice, preventing direct procedures being carried out on live mice.

Project 13	Analysis of skin carcinogenesis in transgenic mice
Key Words	Carcinogenesis, Skin, Transgenic, Mechanism
Expected duration of the project	5 year(s) 0 months

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

Purpose

Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:

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

The incidence of non-melanoma skin cancer [squamous cell carcinoma SCC] is rising steadily. As for all cancers, skin tumour development is a multi-step process and for patients, the most significant stage in clinical terms is the conversion of benign tumours to malignancy and their subsequent increased risk of becoming metastatic. Whether a specific benign tumour possess the potential for progression is unclear, as this depends upon a complex combination of the types of genes mutated and precisely when they are acquired; as each mutation creates a unique tumour context with differing malignant potential. This cancer causing mechanism is also pitted against efforts of protective systems that have evolved to resist the cancer causing process [carcinogenesis] at each stage. To study this problem, genetic engineering technology has been coupled to the classic mouse skin model of multistage carcinogenesis, a mainstay of cancer research for more than 100 years, to create transgenic mice that develop cancer but only after treatment with topical steriods. The model is designed to assess the effects of multiple, genetic insults in well-defined, precancerous stages through to malignant tumours.

The approach exploits genetic engineering techniques so that activation of cancer causing genes or inactivation of cancer preventing [tumour suppressor genes (TSGs)] occurs exclusively in the epidermis of skin. Further, an on/off gene switch system is incorporated into the transgenic mouse design, to elicit highly localised disease but only following topical treatment with a steroid. By testing key genes thought to be responsible for causing tumours [the “driver” mutations] and studying their co-operation, the stage at which mutations become active can be identified.

The genes chosen for analysis represent commonly mutated tumour suppressor genes that help regulate skin cell growth and correct differentiation; and provide the

protective systems that normally cope with mutations occurring naturally e.g. from daily sun exposure.

On going analysis of these multi-gene transgenic mice, will aid in identifying the relevant molecules involved in tumour progression or its inhibition. This approach may highlight a potential Achilles heel appropriate for targeted therapy, equally applicable to other cancer types.

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)?

It is envisaged that analysis of how the progression mechanism unfolds in a real life situation will identify changes to additional key genes to aid in the design of novel therapeutics; whilst the animals themselves may provide a future technology platform able to stringently test novel generic therapeutics or those directly tailored to the actual causal mutations underlying cancer formation in both skin and be relevant to other tissues such as breast and colon carcinogenesis.

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

GA mice; up to 7000 over 5 years.

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

Certain transgene(s) expression elicits a persistent thickened dry, scaly skin in adult mice. The hair is also soft, sparse and shortened. At localised sites [e.g. ear tip] treated with the inducing hormone; wart-like benign tumours appear. Changes in benign tumour size may lead to malignant conversion: typified by a change from rounded, cauliflower–like appearance of papilloma clusters, to a solid, uniform tumour with a typical concave indentation. This is the end point; unless contra-indications apply e.g. benign tumour size, skin location which impedes natural functions. The majority of mice [>90%] are biopsied following humane killing for genetic analysis. Such adverse effects would be closely monitored to adhere to the current recommended guidelines (Workman et al 2010. BJC; www.NC3Rs.org.uk).

Application of the 3Rs

Replacement

The objectives of this project cannot be achieved with out the use of live, geneticly altered [GA] animals.
It is the goal of the project to establish inducible transgenic mouse skin models to verify causal roles for multiple, relevant mutations that drive stage-specific tumour progression, explore the subsequent mechanism and identify putative compensatory sentinel systems that have evolved to inhibit carcinogenesis. Unlike cell culture

systems, transgenic mice offer the possibility to determine the influence of factors critical to malignant progression such as blood supply, an intact immune system, hormonal and cell-mediated growth controls, together with the physical barriers that inhibit tumour cell growth and invasion. However, experiments will utalise skin cells from transgenic mice for tissue culture experiments and the results will be compared to compared to the mouse data. This will asses whether this approach acccuratly mimics tumour progression sufficiently well to replace the need for monitoring malignant progression in adults.

Reduction

Considerable experience has been gained in previous experiments. This monitoring permit experiments to be terminated as soon as significant data has been obtained, thus minimising suffering whilst obtaining meaningful data. Whilst generating these cohorts requires breeding of multiple GM lines, breeding strategies are carefully structured and rigorously monitored to ensure that only the minimum numbers are generated. In addition, given the requirement for topical steroid treatments, animals carrying multiple target transgenes can be routinely maintained; again reducing overall numbers. Untreated breeders act as additional controls and since several genotypes have already been characterised repeating these comparison controls is unnecessary; thus further reducing numbers required.

Refinement

Ideally a transgenic mouse model system should be able to assess multiple, stage-specific genetic insults and accurately mimic the discrete tumour pathologies observed in human carcinogenesis; yet be designed to minimise disease wherever possible. Mouse skin is an ideal target tissue being the classic model for multi-stage carcinogenesis. Here a major advantage of skin models is their accessibility, which not only facilitates induction of localised disease but also allows macroscopic observation of cancer causing events without invasive procedures. A major strength employs a skin-specific inducible gene switch system that prevents disease during animal development or unnecessary disease in internal organs. Thus, during breeding and juvenile development, tumours do not appear; and moreover, as disease can be highly localised [e.g. treatment of ear tip] suffering is minimised.

Project 14	Imaging Studies of Cancer
Key Words	Tumour, Imaging, Therapy
Expected duration of the project	5 year(s) 0 months

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

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);

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

The purpose of this project is to utilise ectopic, orthotopic, genetically-engineered mouse and patient-derived xenograft tumour models to identify imaging biomarkers that accurately report on the pathology and processes relevant to a particular cancer type *in vivo*, and establish their utility in imaging-embedded pre-clinical trials of established and novel cancer therapeutic strategies against these processes.

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

There is an increasing and significant need for appropriately validated diagnostic techniques to characterise tumour pathophysiology accurately for selection of the appropriate therapies, and enable rational and expedient scheduling of combination therapies safely and effectively for each individual patient. Unlike histopathological assessment, follow-up data at multiple timepoints can be obtained in the same animal and same patient, with benefits both in terms of numbers of animals used and size of clinical trials. The non-invasive imaging methods used in this project have either achieved, or are intended to be translated, for clinical use, providing important new biomarkers for use in planning individualised patient treatment protocols, and which have been optimised to also include potentially critical normal tissues.

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

The project licence will primarily involve the use of immune-compromised mice, the simplest species in which tumours derived from human tumour cells can be grown. A limited number of investigations will utilise inbred rats. Over a 5 year period, we anticipate using no more than 2600 mice and 50 rats.

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

All the procedures described in this project licence are designated as moderate. Adverse effects are related to the propagation and growth of tumours, surgical resection of primary tumours, anaesthesia for non-invasive imaging, and the effects of therapy. All animals will be humanely killed by a Schedule 1 method at study end.

Application of the 3Rs

Replacement

Cancer is a disease of unmet clinical need, with 150,000 deaths annually in the UK, so the benefits of cancer research are very clear. Whilst alternative methods of performing cancer research are available, mainly through the use of cultured cells in the laboratory, there are a number of questions in cancer research that can only be addressed using solid tumours in animals. Investigations of tumour blood vessel development, drug delivery and treatment response can only be addressed using pre-clinical animal tumour models, which take into account the tumour-host interaction, the complexity of tumour microenvironment i.e the role of accessory cell types within a solid tumour, and the three-dimensional architecture and function of a tumour. It is not ethical and often not possible to test new treatments on patients without first establishing that they are both effective and safe in animals.

Reduction

Non-invasive and longitudinal imaging methods can refine, replace and reduce the number of animal procedures that are undertaken using traditional histopathological techniques. Non-invasive imaging can:

i) refine procedures in which alternative endpoints can be obtained that represent the active-live process disease state
rather than the animal being killed and an artificial endpoint measured,
ii) replace traditional histology for the assessment of disease progression with anatomic and functional endpoints,
particularly with orthotopic and transgenic models, and,
iii) reduce the number of animals by using each animal as its own control,
harnessing statistical power through the use of

paired statistical tests, and information that may relate better to that observed in clinical assessment of therapy efficacy.

For any study, appropriate group sizes will be determined by power analyses, on the basis of non-parametric statistical tests and a minimal experimental power of 90%, and that an effect size of >30% inpre-clinical studies translates to a potentially efficacious clinical treatment, in addition to any extant literature data.

Refinement

Mice without a fully functioning immune system will be used in order to allow the growth of solid tumours derived from human tumour cells i.e. to prevent rejection. Animals will be housed in cages with sterile bedding, food and water, and also with cage toys and/or nesting materials to provide environmental enrichment. Trained and competent personnel that have experience with pre-clinical models of cancer and are familiar with effects of anti-cancer drugs on rodents will perform all procedures involving animals. Where possible, studies will be designed to achieve several objectives using the minimum number of animals. Animals will be inspected daily and, if necessary, advice will be obtained from a Veterinary Surgeon who is on call at all times. Tumours will not be allowed to grow to a size that causes discomfort, and any animal in which a tumour reaches this size will be humanely killed. Anaesthesia and analgesia will be used to minimise stress and suffering during and after procedures.

Project 15	Metabolism as a novel target for cancer therapy
Key Words	cancer, metabolism
Expected duration of the project	5 year(s) 0 months

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

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);

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

Metabolism of tumours has long been known to differ from metabolism of normal tissues. Different tumours have different metabolic activities that may be defined by their genetic profile, their origin from different tissues and their interaction with other body parts and systems. With this project we are seeking to understand how exactly different tumours use nutrients differently and if they have any specific metabolic vulnerabilities that can be exploited as potential therapeutic targets.

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

This project will substantially increase our knowledge on the regulation and role of various metabolic pathways in healthy and tumour tissues. It can also result in designing novel anti-cancer therapeutics and identifying groups of patients who will benefit from these therapies.

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

30000 mice

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

In a lot of cases the animals will develop tumours, which can be associated with weight loss (cachexia), laboured respiration, enlarged lymph nodes or spleen, and signs of discomfort or condition loss including emaciation and slowing down of the normal activity. However the procedures will never exceed the moderate severity level. At the end of the procedure the animals will be killed by Schedule 1 and the tissues will be used for analysis.

Application of the 3Rs

Replacement

We are seeking to identify how tumours rely on different nutrients and metabolic pathways in the context of a whole intact animal. These settings allow recapitulating the parameters that are naturally influencing tumour metabolism, including a cross-talk between cells composing a tumour and between tumour and other organs. Therefore these settings are the closest to human clinical studies and the results obtained in these settings should have the highest impact.

Reduction

As always we will perform very extensive literature search and analysis of publicly available genomic data sets to guide our choice of models and manipulations.

The efficiency of animal usage is maximised in consultation with animal technicians by careful control of breeding to match research needs with respect to numbers, phenotypic uniformity and health.

We will use statistical approaches to quantify the number of animals required to obtain statistically significant results. Or we will use the minimum number of animals to provide an adequate description, generally on the basis of previous experience (our own, or from the literature).

We will make optimal use of several tissues, fluids and cell types per individual mouse and will provide the other affected tissues to appropriate scientists, so that they do not have to breed mice specifically for their experiments.

Refinement

Mice have been selected for the majority of this work as it is an appropriate model for providing insights into human diseases and it is the species in which reliable transgenic and knockout technologies are most advanced. Wherever appropriate, we will minimise the adverse effects by doing genetic manipulations in specific tissues and in inducible manner.

To minimise stress during breeding and maintenance, we will follow best practice guidelines and follow local refinements of husbandry.

For all manipulations we will adhere to local and national guidelines that aim to minimize suffering. If there is insufficient information available, new manipulations will be pre-screened in small-scale pilot studies to obtain indications of the minimum dose and exposure time that is likely to be effective, thereby minimising any potential suffering.

In all surgery, analgesia will be provided according to contemporary best practice and advice from the NVS/NACWO. Good aseptic surgical techniques, heat & fluid therapy will be provided as necessary. For each protocol where there are a number of optional steps, typically one or two steps will be used and the maximum number will be strictly limited to a maximum of five.

Project 16	Targeting the mechanistic drivers of lung tumour progression
Key Words	Tumour progression, Therapy, Lung cancer
Expected duration of the project	5 year(s) 0 months

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

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:

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

The overall aim of this project is to characterize the crucial steps that normal cells undergo in order to become malignant tumours. Each of these steps may potentially act as an “Achilles heel” for tumour development and our goal is to determine whether tumours can be prevented or treated using drugs that block those progression steps. Our research is particularly focused on lung cancer, the most lethal malignancy worldwide, and for which little progress has been made over the last decades in terms of survival. Over the last 5 years our lab defined key changes that take place during the progression of lung cancers. Our future research aims to use mouse tumour models to better interrogate how the changes we identified may influence tumour development and therapy. Since mice have a similar physiology to humans and can develop lung tumours comparable to those seen in humans, we hope that our findings can help our understanding of the human disease. Furthermore, while our lab carries out basic research we ensure that our findings are of relevance to the clinic by validating the potential relevance of our data and proposed therapeutic targets on human tumour samples.

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 is a basic research project and as such, our immediate goals are to improve our understanding of lung cancer development and to identify novel strategies to efficiently target tumours in the laboratory using relevant models (including mice). However, our work also has a strong “translational” (from basic science to the clinic) component as we focus only on processes or mutations for which we find relevant evidence of disruption in human

cancer samples. The ultimate aim of our work is to contribute to the identification, characterization and pre-clinical validation of novel lung cancer therapeutic targets (e.g. tumour mutations). Once such targets are identified, novel therapies, aimed at killing the cells that carry them, can be proposed for the improved treatment of lung cancer patients. Furthermore, this study may contribute to the identification of biomarkers (molecular signals) of lung cancer that will help us diagnose both early and advanced disease more efficiently.

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

We expect to use 8000 mice over the 5 years of this licence. These will be distributed as follow: Year 1: 1450 Year 2: 1450 Year 3-5: 1700/year The average of mice/year is similar to our current usage and the values calculated overlap with our current breeding/experimental distribution (60/40%). Of note, our colony is currently being moved to a new facility, so we expect to use slightly lower numbers of experimental mice over the first 2 years, as we focus first on colony re-establishment. As tumour development is a slow process, experimental cohorts will gradually increase over time (we require 6-10 months old mice for most experiments). In the last 3 years of this licence we expect to be fully functional and the increase shown in number of mice should reflect this.

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

The majority of the mice being used is expected to suffer no, or only mild adverse effects (~60-75% based on data from previous years). These will be mice used on breeding protocols or for tissue sampling prior to the onset of any health issues. Since this is a project aimed at increasing our understanding of cancer and its therapy, a significant proportion of mice (<40%) will nevertheless undergo tumour induction protocols (typically lung cancer). Importantly, while this process is potentially associated with adverse effects, the majority of these mice will be humanely killed before clinical signs are observed, at stages when only small lung tumours are present. Some animals will nevertheless develop symptoms, but given the extensive expertise of our lab with the models proposed, we are able to identify signs of ill health early and such animals are culled by a humane killing method before those symptoms exceed a moderate severity level, and tissues collected for further analysis. Of note, each new researcher working under this licence receives 1:1 training to recognise these symptoms promptly and lab members carry out extra screenings/week on tumour mice, besides those already ensured daily by facility staff. This approach has enabled us to virtually detect every lung tumour bearing mouse before the onset of moderate signs of distress.

Application of the 3Rs

Replacement

Our work focuses on lung tumours that carry two frequent lung cancer mutations (genetic changes) that cannot currently be treated with available drugs. We have recently shown that the presence of these mutations in tumours causes unique cell behaviour changes, and these changes in turn may potentially be targeted by existing drugs. Therefore, our work is proposing alternative therapeutic strategies for the treatment of a group of tumours that is currently very difficult to treat. But since cancer is a complex disease, characterised by abnormal interactions within cancer cells and between these and surrounding normal cells/tissues, this type of results can only be achieved using models that closely mimic human tumours *in vivo*, as well as the tumour environment (i.e. human body). Mouse models are uniquely advantageous as they not only enable us to closely reproduce the human disease (e.g. lung cancer) but are also highly amenable for research, thanks to their small size, ease of breeding in captivity, short lifespan, extensive physiological/molecular similarities to human and extensive availability of research tools for their genetic manipulation.

Reduction

While animal use is an essential part of this study, it will be minimized in several ways. When adequate, we will use alternative models and particularly tissue culture (primary cells, cell lines and organoids – “mini-organ” structures grown *in vitro*) and human tumour sections. *In vitro* work will be crucial for the initial validation of candidate mechanisms of tumour progression and for testing novel potential therapies. These studies will also enable us to efficiently “short-list” only the most promising findings for *in vivo* validation. In all *in vivo* studies, we will use the smallest possible experimental groups needed to generate data of statistic relevance. We will also use non-invasive imaging techniques, which allow us to follow tumour responses *in vivo* at multiple time points using a single animal. Taken together, these approaches significantly diminish the number of mice required for each study. Finally, most of our mouse lung cancer models develop multiple tumours, allowing for a large number of tumours to be studied using a relatively small number of mice. But importantly, most tumours are relatively small and therefore, in the large majority of cases, this tumour load does not have a major health impact on the animals, and they will be humanely killed prior to the onset of clinical signs.

Refinement

To our knowledge, mice are the only species where human lung cancer has been successfully reproduced, and are therefore the best suited model to answer the relevant scientific questions we propose to ask. Several steps will be in place to minimise suffering throughout this study. Firstly, we always ensure that the mildest severity *in vivo* approach is used in all studies. Secondly, all mice will be monitored for signs of distress, pain, injury or discomfort (such as changes in behaviour, appearance, feeding, weight and tumour size) on a daily basis. This approach enables us to detect any signs of disease early, at which point the animals will be humanely killed. However, in the majority of cases we expect to intervene before that stage, as our knowledge of the models combined to imaging techniques, enables

us to accurately estimate the presence of small tumours prior to their visual manifestation. Thirdly, invasive procedures (i.e. where the body surface is penetrated; ranging from a needle prick to surgery) will be carried out in a manner designed to minimize the discomfort of the animal, using anaesthetics and analgesics, as appropriate. Finally, mice will be housed in small groups to provide social support, but over-crowding avoided to reduce animal stress and to keep breeding efficiency high.