Animals (Scientific Procedures) Act 1986

Non-technical summaries for projects granted during 2014

Volume 14

Projects with a primary purpose of: Basic research - Oncology

Project Titles and Key Words

1. **Identification and validation of novel therapeutic targets for human cancer**
    - Oncogenes/tumour suppressor genes/immune system/small molecule inhibitor/catechins

2. **Significance of alternative slicing in vivo**
    - Tumours, metastasis, splice factors

3. **Controlling CD8+ T cell-mediated immune responses to tumours**
    - Cancer Tumour Immunity

4. **Pathogenesis of Avian Oncogenic Viruses**
    - Marek's disease, Avian Leukosis, Lymphomas, Reticuloendotheliosis, multiple infections

5. **Studies on cancer cachexia and obesity/type II diabetes**
    - Cachexia, tumour factors, weight loss, diabetes

6. **Studies on urological cancer**
    - Prostate cancer, bladder cancer, kidney cancer

7. **Brain metastasis: Imaging and Inflammation**
    - Brain; tumour; imaging; inflammation; metabolism

8. **Role of inflammation in pancreatic cancer**
    - Cancer, Inflammation, Tumour Microenvironment, Pancreas

9. **Generation and characterization of human derived tumour xenografts or PDXs**
    - Breast cancer *in vivo* expansion engraftment

10. **FAP+ stromal cell response to biological stress**
    - Cancer, lymphocytes, cachexia, metabolism, development

11. **Metabolic Dysfunction in Cancer Progression**
    - mitochondria, autophagy, CAFs, oxidative phosphorylation, caveolin

12. **Ribosomal and metabolic stress responses in cancer**
    - Ribosome, metabolism, cancer, therapy, stress

13. **Signalling Pathways in Physiology and Oncogenesis**

- Genes, Cells, Signalling, Development, Cancer

14. Targeted mouse models for pre-clinical studies

- Genetically engineered mouse models, Cancer, Targeted therapeutics, Pre-clinical

15. Targeted Therapy for Cancer

- Cancer, Gene therapy, Radiation, Radiosensitiser, Virus therapy

16. Temporal analysis of cancer evolution in vivo

- Cancer, Biomarker, Therapy

17. Manipulating cellular and microenvironment crosstalk in chronic inflammation and cancer

- Lung cancer, inflammation, microenvironment

18. Gene function in tumorigenesis

- Cancer, colorectal cancer, drug targets, targeted drug delivery, diagnosis

19. Biochemical investigations of the heart

- Heart failure, metabolism, mitochondria, contractile dysfunction

20. Analysis of cancer gene phenotypes

- Oncogene, tumour suppressor gene, tumour formation

21. Genetic determinants of cancer metastasis

- cancer, metastasis

22. Bispecific antibodies for use in cancer therapies

- Cancer, immunotherapy, bispecific antibodies

23. Diagnosis and Therapy of Gastrointestinal Cancer

- Cancer, Abdomen, Optical Imaging

24. Action of and intervention in control pathways in cancer

- Kinase, cancer, drugs

25. Investigation of RAS oncogene mutant cancers

- Lung cancer, therapy, early detection

26. Investigating cellular protein production

- cancer, protein production, gene expression.

27. **The phosphoinositide-network in health and disease**
    - Inflammation, cancer

28. **Understanding the molecular basis for invasion and metastasis of melanoma and pancreatic cancer**
    - Cancer metastasis, pancreatic cancer, melanoma

29. **PTEN and the PI 3-kinase signalling pathway**
    - Cancer, signalling, pten, tumour suppressor

30. **Cancer biology including host immunity in zebrafish**
    - Cancer, immunity, zebrafish

31. **The Tumour Microenvironment in Cancer Progression**
    - Cell death, Inflammation, lymphoma

32. **Mechanisms of normal and leukaemic haematopiesis**
    - Haematopoietic stem cells; leukaemia; haematopoiesis

33. **Understanding the repair of damaged DNA**
    - DNA damage; repair; cancer; chemotherapy

34. **Mechanisms of cancer development**
    - Cancer, heterogeneity, stem cells, epigenetic

35. **Development and refinement of Small Animal Imaging**
    - Imaging, Development, Biomarkers

36. **Novel therapies for malignant germ cell tumours**
    - MicroRNA, germ cell tumour

37. **Molecular Imaging of Cancer**
    - Imaging, cancer, instrumentation, contrast

38. **Genetics and treatment of acute lymphoblastic leukaemia**
    - Acute lymphoblastic leukaemia, measles virus

39. **PML and its network in tissue development and disease**
    - Cancer, stem cells

40. **Models of lymphomas to identify therapeutic targets**
    - Lymphoma, microenvironment, targeted therapy

41. Targeted molecular and immune therapies for cancer

- Cancer, melanoma, immune response, inflammation, immunotherapy

42. Regeneration and cancer in epithelial tissues

- Stem-cells; Regeneration; Tumour; Epithelial tissues

<table>
<tr><td>PROJECT 1</td><td>Identification and validation of novel therapeutic targets for human cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Oncogenes/tumour suppressor genes/immune system/small molecule inhibitor/catechins</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>In previous studies we have gathered evidence that specific genes such as frizzled receptor 6, MYCN and EZH2 are increased in neuroblastoma or breast cancer. Other genes, such as PML or ApoJ/clusterin are decreased. In other circumstances, specific cells of the immune system are altered in cancer, resulting in reduced antitumour immunity. The main objective of the project is to reproduce the alterations in gene expression observed in human disease (i.e. neuroblastoma and breast cancer) in a mouse system in order to verify the causative role of gene loss or gain. A further aim of the study is to modify the activity of the cancer genes using pharmacological intervention to model therapeutic approaches that could be later translated in the clinic.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The likely benefits are i) an increase of our understanding on how the process of tumourigenesis occurs in a living animal and ii) the development of less toxic therapeutic approaches that are more specific and effective than traditional chemotherapeutic drugs.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Mice, about 1000 in 5 years</td></tr>
</table>

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will i) breed mice with genetic modifications that renders them prone to a cancer similar to that of the human counterpart. We will not allow mice to carry on with full developed tumours, but we will kill them in a humane way before sign of suffering will develop; ii) we will inject mice with a defective immune system with human tumour cells to assess the efficacy of drugs in a living context.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Assessing the effects of human oncogenes/tumour suppressor genes and their genetic and pharmacological manipulation in the context of mouse models is essential to investigate the host-tumour interaction, which is an important part of cancer growth and cannot be adequately investigated in vitro.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In each of the experiments described in this PPL the mice will be killed at the earliest sign of discomfort or abnormal behaviour or other adverse effects, well before the metastatic spread has resulted in the growth of very large tumour masses. We will take advantage of imaging systems that enables reduction of mice numbers since tumour growth can be quantified at different time points in the same animal.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the most suitable species because there are already many murine models of human cancer in existence, because the immune system is highly analogous to the human system, there is high degree of structural homology between human oncogenes and their murine counterparts and because they are a species of relatively low neurophysiological sensitivity but in which extent and effects of tumour growth is readily measurable. Suffering will be minimised by using small gage needles for injections and daily assessment of the welfare of mice by specialised personnel in the animal facility.

PROJECT 2	**Significance of alternative slicing in vivo**	
Key Words (max. 5 words)	Tumours, metastasis, splice factors	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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)	Despite tremendous efforts in biomedical research in the last 50 years there is still a high incidence and mortality due to cancer in the world, including UK. More than 90% of deaths related to cancers are attributed to metastasis — the process through which the cancer spreads from the initial site to various organs. Though many treatment options are available these days for cancer patients we are still far from curing the disease and most often we are barely able to slow its progression but not prevent people from dying of cancer. Therefore there is a lot of interest in *i)* understanding more of the basic biology of cancer and metastasis; and *ii)* use this basic information to find novel therapeutic targets and avenues.	
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 addresses one of the most under-explored area in cancer cell biology — the possibility to manipulate defective cell properties at a different level of regulation than the ones used today iri anticancer therapies. This is the level of so-called “post-	

	transcriptional" regulation in which a class of molecules named splicing factors play a major role. While a lot is known about the properties of splice factors in cell culture there is very little understanding to whether manipulation of splice factors is able to inhibit tumour growth in vivo as well as the spread to different organs. Therefore the research described in this project is essential for obtaining proof-of-principle that splice factors and their regulators may be used as therapeutic targets to fight cancer.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice — approximately 860 mice over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Typically we will implant tumour cells (derived either from mice or in some cases from man) into mice; this involves only a single injection either under the skin or by microsurgery into the prostate gland, breast or under the kidney capsule. We will then monitor the animals carefully, and make use of special imaging machines that allow us to repeatedly view the tumour cells in the living animals both in the primary tumour as well as during their spread in the organism. This is performed under anaesthetic (to keep the animals still) and is non-painful. This technique allows us to reduce the number of animals that we need to use. Animals may also receive substances which are intended to alter tumour growth. These may be given by a number of routes, but often can be given in water or food. Careful monitoring of the animals is crucial for our studies, and we have strict criteria when animals are to be killed, depending upon the level of development of tumours, For example, if the skin over a tumour was seen to breakdown, the animals will be killed straightaway. Animals will be inspected daily and the presence of signs of distress assessed. Tumour will only be allowed to grow to a predetermined size and if the animals

	normal behaviour is altered or they have abdominal distension they will be killed. The design of our experiments will be such that we will minimises the number used.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Tumours are as complex in structure arid organization as organs are. Though the cancer cells form the bulk of the turnours volume they contain other types of cells e.g. inflammatory cells as well as a sophisticated vasculature. An important component of the ability of tumours to grow is based on the interactions with the host organism arid the structures surrounding them (so called tumour microenvironment). Therefore it is essential to study tumour biology in vivo in animal models as only limited information may be obtained from culturing cancer cells in incubators (The only other alternative). Additionally, metastasis, the process of cancer spreading to other organs, is a process that happens in vivo in the whole organism and there are no other experimental alternatives.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments will be done using a special design with repetitive measurements of the tumour volumes in the same animal. This has greater statistical power, and animals need only be killed at the end of experiments rather than at each time- point drastically reducing the numbers of animals being used. Transgenic (genetically altered) mice numbers will be kept to a minimum by using crossing designs that result in minimal animal numbers, demand will be assessed before breeding and crossing, colonies will only be maintained while there is an experimental plan and funding allocated.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having	Two animal models will be used: 1. Nude mice — mice that have been genetically altered to inhibit their immune system; these mice are widely used and considered the best model across the world for

regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	human cancer cells implantation studies; the main reason is that human cancer cells would not be able to grow in a different species if the immune system would be intact 2. Genetically altered mice — compared to other species mice are considered the easiest to be manipulated genetically. We are using mouse tumour protocols that have previously been used to study growth inhibition to reduce the number of experiments. Thus mice will be killed before the tumour load becomes large enough to impair health in these animals, thereby reducing the likelihood of pain, suffering, distress or harm. The introduction of repetitive imaging procedures reduces the number of animals that need to be used for tumour experiments, and reduces the burden on those animals. Tumours can be detected when smaller than palpable, and metastases can be imaged before signs of distress occur. Since the objectives of these experiments are to determine the mechanisms underlying splice factor importance for tumour growth and metastasis in animal models of disease we will be investigating the early time points of tumour growth, when the least adverse effects are seen. Therefore these experiments are designed to cause the least pain, suffering, distress or lasting harm possible to achieve the objective. Furthermore, if the animals appear to be suffering, in pain or the tumours show evidence of harming the animal, the experiment will be terminated by killing the animal.

<table>
<tr><td>PROJECT 3</td><td>Controlling CD8+ T cell-mediated immune responses to tumours</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cancer Tumour Immunity</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Regulatory use and routine production</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Preservation of species</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Higher education or training</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td><s>Yes</s></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Cancer is still one of the main causes of death worldwide. Around 325,000 people were diagnosed with cancer in 2010 in the UK. Every two minutes someone in the UK is diagnosed with cancer and more than 1 in 3 people in the UK will develop some form of cancer during their lifetime. Most cancer treatments currently in use rely upon the surgical removal of tumour tissue; chemotherapy, which works by targeting DNA synthesis and cell division; and radiotherapy, which aims to kill tumour cells using ionising agents. However, there is a necessity to find new ways to target tumours due to difficulties in surgery, and the localised toxicity and lack of specificity of conventional chemo- and radio-therapeutic approaches. We believe that therapeutic approaches that produce anti-tumour immune responses may provide us with new ways to treat cancer without damaging the rest of the body. However, the major problem is that as tumours</td></tr>
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	develop they often create a highly immunosuppressive microenvironment that is able to switch off the cells in the immune system that have the ability kill the tumour cell. However, it is still not clear how the tumour is able to cause such immune suppression.
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)?	With an ever-increasing aging population the incidence and the prevalence of many cancers is increasing. Currently, most treatments rely upon combinations of surgery chemotherapy and radiotherapy. However, there is a necessity to find new ways to target tumours due to difficulties in some surgeries, and the toxicity and lack of specificity of conventional chemo- and radio-therapeutic approaches. The immune system is able to specifically target and kill tumour cells with minimal non-specific collateral damage. However, a major problem is that larger tumour masses create a very immunosuppressive environment which allows them to progress unhalted. This work will provide us with an understanding of the mechanisms of tumour-mediated suppression of immune responses to help ensure the success of future anti-cancer immune based therapies.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will require us to use a unique genetically modified strain of mice as a source of tumour specific immune cells. Thus we anticipate using around 1500 mice in total for the duration of the project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Part of the project requires the breeding of genetically modified mice with specific alterations to the immune system. We breed the mice in highly protected environments to avoid health problems. In studies of tumour growth, the mice may experience moderate effects from a combination of expansion of a tumour, blood sampling and administration of substances that may modify immune responses.
Application of the 3Rs	
1. Replacement	Despite maximum effort to carry out ‘Replacement’ strategies using cell cultures systems, the

State why you need to use animals and why you cannot use non-animal alternatives	complexity of both the host immune system and the influence it has upon tumour growth cannot be effectively replicated *in vitro* and consequently conclusions drawn using such systems need to be tested *in vivo*.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We reduce the numbers of animals used by doing as much work as possible using tissues obtained from un-manipulated mice. In many of our experiments animals are used only for the generation of primary tissue cell cultures and hence invasive procedures are avoided. However, the clinical study of cancer pathogenesis and prevention requires the manipulation of experimental animals as subjects and we are confident that our advances in cancer therapy justify the use of animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The use of an experimental mouse model is based upon the following: i) immune responses in mice are very well-understood and correlate with human immune function, ii) there is a greater range of mouse-specific reagents, and iii) the use both conventional inbred and numerous transgenic inbred mouse strains that serve as donors and recipients minimises variability in the responses between individuals; thus ensuring that fewer animals are required as a result. We have continued to refine our model such that we have consistently reliable control groups with which to compare our experimental groups, ensuring we generate reliable and robust data to minimize the need to repeat experiments; other than that which is necessary to provide statistical significance. Similarly, we have consistently reliable tumour cell lines, all of which grow subcutaneously as a clearly defined non-invasive circumscribed mass without spontaneous metastases. This allows us to make accurate measurements of tumour growth and allows us to clearly define and monitor experimental end points. Importantly, for all tumour growth experiments we will adhere to and commit to working within the guidelines on tumour growth in animal models, as set out by the UK National

	Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3R), [http://www.nc3rs.org.uk], and in the guidelines for the welfare and use of animals (British Journal of Cancer. 2010 May 25; 102(11) 1555). We always question whether or not the potential benefits justify any suffering that the animals may endure. Whenever experiments are performed which involve any degree of distress or potential pain, we routinely check the procedures in order to 'Refine' them further for future studies so that these effects may be minimised. This will continue to be our policy in the future.

<table>
<tr><td>PROJECT 4</td><td>Pathogenesis of Avian Oncogenic Viruses</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Marek’s disease, Avian Leukosis, Lymphomas, Reticuloendotheliosis, multiple infections</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>Five years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
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<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
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<tr><td>Higher education or training</td><td></td><td>No</td></tr>
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<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Cancer is a complex multistep disease involving multiple cell types. Unlike in human health, the vast majority of cancers are induced by viruses. In poultry, the major virus-induced cancers are Marek’s disease, avian leukosis and reticuloendotheliosis, all of which affect different types of blood cells. Dynamic processes in the pathogenesis of cancer can only studied in vivo as there are no in vitro models that can capture all the complexities of the disease. We have developed very good systems to manipulate the genomes of the avian oncogenic viruses, which would allow us to develop viruses that express fluorescence markers, which would allow us to track the infections in vivo using advance bioimaging tools. In this project, we will use infection models of these diseases using modified viruses expressing makers in their natural avian hosts to unravel the dynamic</td></tr>
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	molecular events that lead to cancer. **The objectives of the project are to** (a) determine the pathogenic mechanisms and transmission dynamics of avian oncogenic viruses in the target avian hosts, (b) examine the host immune responses against these viruses or vaccines derived from them to identify key mechanisms that would help improving the control strategies, (c) generate novel recombinant vaccines that can induce protection again other major avian viruses, and (d) study the *in vivo* dynamics of these viruses using in new imaging technologies.
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 license will help to build on the findings on the pathobiology of the diseases caused by the three major avian oncogenic viruses. The project license will help to unravel the factors that are involved in the induction of diseases, to analyse global changes in gene expression profiles to identify specific pathways associated with induction of tumours by the viruses. The project license will also give the opportunity to examine the role of vaccines in driving virulence of the viruses. Finally, successful development of novel recombinant vaccines will help to control major avian diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	Most of the work is planned in poultry. Approximately 6000 birds is expected to be used during the 5 years of the project, in addition to small number of rabbits and mice for generating specific antibodies against virus and host proteins.
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 adverse effects from the procedures described can vary, as listed under each of the protocols. The expected maximum severity level for the various procedures is thought to be moderate. Majority of the animals will be killed by a schedule 1 method, although on some occasions, non-schedule 1 methods may also be used.
Application of the 3Rs	
1. Replacement	Cancer is a highly complex, multifactorial, multistep dynamic process involving several cell types and

State why you need to use animals and why you cannot use non-animal alternatives	events. There are no complete *in vitro* models that can simulate this complexity. Hence there are no non-animal alternatives that can completely replace the use of birds. Similarly, the immune responses to these diseases can also be effectively studied only in an infected bird, again due to complex nature of the responses.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Most of our experiments are carried out using well characterised viruses using chicken lines with limited heterogeneity, allowing us to use minimum number of birds in different groups. Moreover, our long experience in these disease models will help to decide on the numbers needed. The group sizes and the replications will be kept to the minimum possible to achieve statistically robust data.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We will carry out refinement in a number of areas. First, we will use more robust clinical scoring system to calculate the humane end-point (see appendix 4) as the diseases by oncogenic viruses generally progress slowly with milder clinical signs such as lethargy, loss of appetite and progressive weight loss. This will help in early monitoring and removal of birds. Chickens are monitored twice daily and killed by a Schedule 1 method by trained personnel in an ante-room so as not to distress the other chickens in the pens. As a further refinement of the protocol, we would increasingly use chicks derived from vaccinated parent flocks, and the maternal antibodies usually give better protection from early clinical disease. We also propose to refine the MDV transmission experiments from the isolators to the floor pens based on the data from the pilot experiments on floor pens and showed that the data obtained from floor pens were comparable to those from isolator experiments. Chickens used in experimental studies have daily human contact with animal care staff who replaces their food and water, and they become used to handling.

<table>
<tr><td>PROJECT 5</td><td colspan="3">Studies on cancer cachexia and obesity/type II diabetes</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Cachexia, tumour factors, weight loss, diabetes</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td>No</td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Objective 1: The main objective is to prevent weight loss in cancer patients by targeting anti-cachexia therapies at key tumour products therefore improving the quality of life and improving survival of cancer patients.

Objective 2: Obesity and type II diabetes are becoming the major medical problem of the 21st century. From our studies on cancer cachexia we have discovered a fat mobilizing substance called Zinc-α-glycoprotein (ZAG), a natural material, which reduces body fat in obese mice, and also partly controls the on-going diabetes. This work will extend our understanding of how ZAG produces this effect to enable further clinical studies in humans.</td></tr>
<tr><td>What are the potential benefits

Likely to derive from this</td><td colspan="3">The potential benefits are a treatment for cancer cachexia, which is responsible for 25% of all cancer</td></tr>
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project (how science could be advanced or humans or animals could benefit from the project)?	deaths, as well as providing a new therapy for the treatment of obesity/type 2 diabetes, using a material that is naturally found in the body. These studies will also elucidate fundamental metabolic pathways, particular for these disease states, which may provide further therapeutic exploitation, as well as helping us understand how these conditions occur.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse 5800 over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The major adverse effect of the cachexia study will be weight loss, although this occurs without a drop in food and water intake. Any animal losing more than 20% by body weight or showing signs of suffering will be immediately sacrificed. For the obesity study the major adverse effect is diabetes and this will be closely monitored, although the proposed treatment should reduce this and improve the quality of life for the animals. At the end of the study all animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Non-animal alternatives form a major part of this project, particularly for mechanistic studies. However, both cachexia and obesity/diabetes are complex metabolic problems involving many organ systems which can be only partially replicated in vitro, necessitating the need for animals. Use of animals in the past has enabled us to find metabolic pathways that could not have been predicted from in vitro studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	A power analysis will be performed to limit group while still retaining statistical significance, so that experiments will not have to be completely.
3. Refinement Explain the choice of species	Mice are the lowest vertebrate group which suffer cachexia on development of a tumour. Our previous studies with the MAC16 tumour have identified a

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.	component of fish oil as having therapeutic activity, and this has also been replicated in cancer patients. In addition the MAC16 tumour produces a material which breaks down muscle that is also found in human tumours, so attacking this substance should provide a therapy which is also relevant to cancer patients. Welfare costs to the animals will be reduced by prior screening of materials in vitro with only active compounds being tested on animals. In addition the toxicity will be determined on a single mouse prior to larger group testing and suffering will be reduced by regular inspection of the animals during the course of the study.

<table>
<tr><td>PROJECT 6</td><td>Studies on urological cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Prostate cancer, bladder cancer, kidney cancer</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Urological cancers including prostate cancer remain a global health concern. We aim to improve our knowledge of these diseases with an ultimate aim for developing better treatments and tests for clinical application. New models of prostate cancer will direct future efforts in novel targets for therapy and identification of new biomarkers (tests) for aggressive prostate cancer. We will apply our existing models to test the importance of specific genes/pathways in driving disease progression in prostate cancer including metastasis and resistance to treatment such as androgen deprivation therapy and chemotherapy. Hence, research planned in this license may have important clinical implications and can potentially provide the basis for research that can improve clinical practice for patients with urologic malignancies.</td></tr>
<tr><td>What are the potential benefits likely to derive from this</td><td>The likely benefit will originate from knowledge gained from our experiments which will inform</td></tr>
</table>

project (how science could be advanced or humans or animals could benefit from the project)?	ongoing and future efforts in drug development. Data from work carried out within this license will be validated using resources from clinical prostate cancer.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse ~50,000 over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Moderate severity - When cancer develops in the urinary tract, animals may experience abdominal distension or develop urinary symptoms. Animals will be humanely killed at the end of the experiments in accordance of detailed protocol within this licence.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Clinical cancer samples (especially prostate and bladder) are highly variable, and specimens from human have not provided detailed molecular information on how best to develop new treatment.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Cells from our mouse models will be used in *in vitro* culture and also in xenografting experiments, thus reducing the overall number of animals required. The continued use of imaging will also improve our study design and reduce number of animals required to answer specific questions.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mouse model is well established to be of value to study human urological cancer. They can be developed to mirror the exact genetic abnormalities found in clinical cancers. The use of gene-induction to target the organs of interest will greatly diminish the impact on the general welfare of the animal. The use of novel imaging will also avoid invasive procedure to gain important insight into cancer biology and tumour response to treatment.

<table>
<tr><td>PROJECT 7</td><td>Brain metastasis: Imaging and Inflammation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Brain; tumour; imaging; inflammation; metabolism</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Regulatory use and routine production</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td>~~No~~</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The objectives of this project are to understand how the local environment in the brain contributes to the growth of brain tumours. In particular, we are interested in secondary tumours in the brain which arise from primary tumours elsewhere (mainly breast, skin and lung). Currently, it is very difficult to diagnose these secondary tumours in the brain early enough to effectively treat them. At the same time, the specialised barrier between the brain and the blood prevents many potential therapies from accessing these tumours. Again, this reduces the effectiveness of treatment. Consequently, the prognosis for patients with secondary tumours in the brain is extremely poor. Under this project, we aim to identify factors in the response of the brain to the presence of tumours that either help or prevent tumour growth. In this way we expect to identify new targets for therapy. We are also</td></tr>
</table>

	developing new approaches to enabling potential therapies to cross the barrier between the blood and the brain in order to enable effective treatment of brain tumours. Finally, we are developing new imaging methods that we believe will both help to diagnose secondary tumours in the brain earlier and also report on processes within the tumour that may impact on therapy effectiveness, such as vascularity, blood flow, acidity and amount of oxygen.
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)?	Up to 40% of all cancer patients will suffer tumour spread to the brain. Owing to improved treatment of primary tumours, the incidence of secondary brain tumours is increasing. There is currently no cure, and life expectancy after diagnosis is generally only a few months. It is critical, therefore, that we develop new methods for earlier diagnosis and improved treatment. We anticipate that this work will enable development of new therapies and/or allow better application of existing therapies in patients with known or suspected brain metastases. We will also develop imaging methods that we believe will enable earlier diagnosis and more effective therapy in patients with brain metastases.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use 4000 postnatal rats and 6700 postnatal mice in this work over a period of 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The likely incidence of adverse effects resulting from the majority of procedures used is very low, and may include drug toxicity, vascular damage or haemorrhage following injection, haemotoma following blood sampling and incomplete wound healing or infection following surgery. With our tumour models there is a greater chance of either neurological symptoms or compromised health owing to the nature of the models. In all surgical cases it is also expected that animals will experience a

	degree of pain. For these reasons we monitor the animals very closely and always seek advice from the veterinary staff within the University. We use analgesia where appropriate and all surgical procedures are performed under general anaesthesia. The likely level of severity in most cases is mild-to-moderate. At the end of the experiment the animals will be killed painlessly according to an appropriate procedure.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	At this point in time there is no tissue culture or modelling system that can duplicate or predict the process of secondary tumour spread in the mammalian system. We will support our animal studies with cell-based investigation of tumour-brain cell interactions, but this does not fully model the intact mammalian brain where multiple cell types are present in a complex 3-dimensional geometry and are supported by, and interact with, both the circulatory and immune systems. Consequently, whilst such models may allow modelling of specific elements in isolation, they cannot be used to model the entire system and, thus, do not represent a viable alternative to animal models. It is also not possible to fully test new imaging techniques in cell-based or non-biological systems, since the MR signal characteristics are not the same as those obtained from the brain *in situ*. All of our new contrast agents are tested extensively in phantoms and cell-based systems first, and only progress to animal studies once efficacy has been established in our standard cell line assays. Nevertheless, since the overall goal of this work is to develop new techniques and agents that can be applied clinically a period of pre-clinical testing in animal models is essential and unavoidable.

2. Reduction Explain how you will assure the use of minimum numbers of animals	Much of our work involves imaging in animal models, and as a consequence of being non-invasive this allows us to study temporal events in the same animal. Thus, the use of imaging, and MRI/MRS in particular, for animal experiments represents significant reduction. In addition, many different types of data can be obtained from each experiment, which further reduces the number of animals required. The number of animals in this licence has been chosen to be sufficient for statistically reliable data, based on previous results, the intrinsic variability of imaging data, and the magnitude of the expected changes. We consult extensively with the departmental statisticians as new studies begin to ensure that the optimal number of animals is used to obtain meaningful results and also kept to a minimum. Appropriate control groups are included and specified in each protocol and will be essential for proper statistical analysis and evaluation of observed effects. In all cases brain tissue will be used following the *in vivo* experiments, for immunohistochemical and molecular analysis, in an attempt to use the minimum number of animals 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.	Much data is available on the biology of rodents and they are widely used in models of experimental neuropathology. It is recognised that many of the cellular and intracellular events we study in rodents are directly relevant to understanding of the physiology and pathology of the human nervous system. It is not possible to conduct meaningful experiments in this area in anything other than mammals since the development of the central nervous system and immune system is unique to this class. In most cases we will use both rats and mice and we have a substantial body of work in these species on which the current work is based. For the tumour models, we have spent considerable time refining these models to

	allow a longer time span for investigation of the brain tumours in symptom-free animals. Moreover, although a small number of animals will be taken to the latest time points possible in many cases we are more interested in the early stages of tumour development, when the tumour burden is too small to cause distress to the animal. In order to better understand the contribution of inflammatory pathways to brain tumour development we will use various strategies to modify inflammatory pathways in some animals. Wherever possible we will use the most refined approach to modify aspects of the inflammatory response (e.g. up or down-regulation of single effector proteins) in order to minimise both adverse effects and additional suffering in these animals. Indeed this is scientifically important since it allows us to dissect out specific contributions to the process. We work closely with the NACWOs and vets, to establish relevant behavioural scoring sheets for each individual model used. In this way we ensure that all relevant clinical symptoms and signs are assessed and humane endpoints applied appropriately. All surgical and anaesthetic methods will be reviewed regularly as new methods become available.

<table>
<tr><td>PROJECT 8</td><td>Role of inflammation in pancreatic cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cancer, Inflammation, Tumour Microenvironment, Pancreas</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td></td><td>YES</td><td>NO</td></tr>
<tr><td>Basic research</td><td>X</td><td></td></tr>
<tr><td>Translational and applied research</td><td>X</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>X</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>X</td></tr>
<tr><td>Preservation of species</td><td></td><td>X</td></tr>
<tr><td>Higher education or training</td><td></td><td>X</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>X</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>X</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Our main objectives are (a) to understand how leukocytes contribute to the initiation and progression of pancreatic diseases and (b) to investigate whether disrupting the cross-talk of immune cells with pancreatic cells has implication during development or treatment of pancreatic diseases.

Pancreatic cancer is an aggressive malignant disease with a 5-year survival rate of less than 5 %, and unmet clinical needs. During pancreatic cancer progression, cells from our immune system progressively accumulate at the primary tumour site. Although these cells are initially recruited to kill malignant cancer cells, recent studies have shown that in fact the opposite can be true and that our</td></tr>
</table>

	immune cell contribute to the establishment of a tumour promoting microenvironment. In addition, preceding inflammation of the pancreas (pancreatitis) markedly increases the susceptibility to pancreatic 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 the new knowledge about how inflammation promotes pancreatic diseases. This will be of interest to pre-clinical scientists interested in pancreatic digestive diseases and carcinogenesis. We aim to publish our findings in peer reviewed academic journals. The secondary potential benefit relates to the possible identification of new molecular targets which may lead to improved diagnosis and/or treatment of pancreatic digestive diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	All animal experiments will be performed in established mouse models over a period of 5 years. The number of animals used for each treatment will be kept to the minimum required, this being determined by statistical analysis of the results of multiple previous experiments performed myself or by other laboratories for each of the proposed protocols.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	During this project we will test in well-established murine pancreatic digestive diseases models whether inhibiting or modulating of specific immune cell functions affects susceptibility to pancreatic cancer, pancreatic cancer progression, and it's response to standard chemotherapy. At the end, all animals will be humanely euthanized by a Schedule 1 method. Adverse effects will be kept to a minimum to achieve the over-riding clinical scientific objectives described and the majority of studies are expected to be in a mild/moderate severity level.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our animal experiments will be complemented by in vitro experiments using cell lines and short term primary cultures of immune cells and pancreatic cancer and stromal cells. However, this complex interaction between immune cells, stromal cells,

	and malignant cancer cells in vivo mean that all relevant experiments cannot currently be performed in vitro. We will also study fresh and preserved tissue samples obtained from biopsis from human subjects with pancreatitis and/or pancreatic cancer, but for ethical reasons it is not possible to manipulate gene expression in human subjects.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals used for each treatment will be kept to the minimum required, this being determined by statistical analysis of the results of multiple previous experiments performed myself or by other laboratories for each of the proposed protocols.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	All protocols and procedures used in animals will be the mildest possible affecting the minimum possible number of animals to achieve the over-riding clinical scientific objectives described.

<table>
<tr><td>PROJECT 9</td><td colspan="3">Generation and characterization of human derived tumour xenografts or PDXs</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Breast cancer in vivo expansion engraftment</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">We aim to establish a bio-bank of human breast cancer tissue by maintaining live tumour fragments from surgically removed samples. Our goal is to accelerate personalized cancer medicine by profiled genetic changes in primary cancer cells with drug sensitivity.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">By establishing state-of-the art pre-clinical breast cancer models (PDXs) from consented patients in the clinic willing to donate their samples for research purposes, we aim create a unique collection necessary to model the intra- and inter-tumour heterogeneity of breast cancer. This will serve as a reference resource for the research community and will serve as a platform to develop a personalised approach to cancer treatments</td></tr>
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What species and approximate numbers of animals do you expect to use over what period of time?	We might use around 10000 mice, some will be used as breeders and some as hosts for primary human breast cancer samples or patients derived tumour xenografts. We try to engraft clinical specimens from consented patients from the Breast Cancer Unit at Addenbrooke's Hospital. This varies between 0 and 5 samples weekly. Each sample will be engrafted in individual mice. From the ones that successfully engraft, we will expand cells from that individual patient by serially transplanting PDX's tissue into more mice. We aim to keep this project going for as long as possible as big numbers of engrafted tissue samples are needed to achieve our aims.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will follow the guidelines for the welfare and use of animals in cancer research to minimise the adverse effects if any, and perform appropriate humane endpoints when needed. Animals will be killed generally before the tumour size exceeds 1.5cm in diameter, or if the tumour is restricting the normal movement of the animal. Adverse effects might be derived from the surgical procedures, as mice will experience some short-term post-operative discomfort. Other likely adverse effects will be due to toxicity from the anti-cancer drugs/substances we aim to administer to these models for our research purposes. Tumour burden will be limited to decrease adverse effects derived from subcutaneous tumour growth. However, all animals will be monitored daily for signs of ill health and assessed for clinical signs that necessitate intervention. Animals will be killed if they show any signs of ill health likely to exceed the moderate severity limit and showing adverse effects that cannot be ameliorated by mild veterinary interventions.
Application of the 3Rs	
1. Replacement State why you need to use	Patient derived tumour xenografts are the current state-of-the art preclinical models. Cell lines have been proven to be very useful to provide genomic:

animals and why you cannot use non-animal alternatives	phenotype associations yet are a completely artificial system with little/none resemblance to the tissue of origin. Due to the well accepted shortcomings on the use of cell lines, the ideal scenario for preclinical drug testing would be to use primary cells from each individual patient to personalize each treatment decision. We have tried to expand human breast cancer cells *in vitro* but have noticed that they drift from their original nature. By expanding human breast cancer tissue in mice through the generation of PDX models we have noticed that much of the genomic and functional heterogeneity is maintained. PDX models thus are going to revolutionize cancer treatment as they represent the first step towards personalized treatment avenues.
2. Reduction Explain how you will assure the use of minimum numbers of animals	When possible, we will use *in vitro* approaches to generate proof-of principle and PD/PK data that will help us design a pre-clinical study with the appropriate mouse numbers.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We have improved our scientific procedures (use of analgesics, anaesthesia etc.) to minimize any type of unnecessary suffering or distress to the animal. Moreover, the animals are monitored daily.

<table>
<tr><td>PROJECT 10</td><td>FAP+ stromal cell response to biological stress</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cancer, lymphocytes, cachexia, metabolism, development</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>Five</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>1. To understand the interaction of cancers with the immune system, and to discover new methods of cancer immunotherapy.
2. To understand the role of stromal cells in the development and maintenance of organs and tissues.
3. To understand the metabolic and immunological responses to diseases, such as cancer, that may manifest as cachexia and immunological dysfunction.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>1. Improved cancer immunotherapy.
2. New insights into tissue regeneration.
3. Therapies for cancer-associated cachexia.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use</td><td>Mouse: 30,000 over 5 year period.</td></tr>
</table>

over what period of time?	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The expected level of severity of the overall application would be moderate. In all cases maximum effort will be undertaken to ensure that this level will is adhered to. With regards to individual expected adverse effects of specific protocols, study of cancer cachexia may result in significant weight loss, the pancreatic murine model of cancer (KPC) has well described adverse phenotypic characteristics that will be closely monitored. In all cases of tumour study, anaemia, weight loss, ascites, pronounced lethargy and decreased interaction may be observed and will be addressed immediately as they occur to minimise any and all suffering in the mice. At the end of planned procedures, mice will be humanely killed by competent and trained persons working under the project license.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The biological and health-related questions of cancer immunotherapy and the maintenance of tissues and organs cannot be addressed by in vitro methodology. In addition, the capacity to genetically modify mice has enabled the development of models that allow discrete and informative experiments to be performed.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Every opportunity will be taken to decrease the number of animals used for each experiment whilst still maintaining the statistical relevance of the subsequent data. To maximise information, multiple body sites will be examined from each animal and multiple analysis types will be conducted on each sample, where possible. Breeding of animals will be carefully monitored to ensure no excess breeding occurs.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	We will use unique, genetically modified mouse strains that refine our capacity to design and conduct precise experiments that focus on the genes and cells that are most important and

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	relevant to human biology and diseases.

<table>
<tr><td>PROJECT 11</td><td>Metabolic Dysfunction in Cancer Progression</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>mitochondria, autophagy, CAFs, oxidative phosphorylation, caveolin</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>Five years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Our aim is to identify novel metabolic-related cancer markers which can predict recurrence and progression to later-stage disease and metastasis.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The discovery of the two-compartment, energy-metabolite transfer process from the non-cancer to the cancer cells is a novel and important finding. Presently, there are foods and food supplements available that might suppress the effect as we work to discover more effective ways of targeting it.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Our experiments will use mice, we estimate over the five year project period to use 9000 animals.</td></tr>
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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?	Our experiments are simple and straight forward. We implant a small number of cancer cells under the animal's skin. We allow the cells to form a small mass (tumour), which then ends the experiment. We measure tumour growth rates and examine the animal's organs for disseminated cancer cells. Sometimes we will administer drugs to the animals to suppress the growth of the tumours. These drugs are the same as would be given to human patients. Animals used in this project will not be transferred to other projects. Those used in our experiments will be killed at the conclusion of the experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Researchers have discovered over the past decades that many cell types contribute to the growth of Breast and other cancers. These cells can include immune cells, fat cells and fibroblasts which create and are part of the matrix that tissues are composed of and which cancers grow in. Animals more closely model humans than cells in culture and will possess the same array of cell types. To study how cancer grows, invades into surrounding tissues and disseminates to distance organs, we need at times to use a model system which more closely matches the complexity or our own.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Although animal experimentation is highly useful, we take every step to minimize the numbers used. Great care is taken in the design of our experiments to reduce variation, increase reproducibility and reliability, which all results in increased statistical power to our data, thus reducing the size and number of experiments. Additionally, as we more fully understand the cellular relationships within tumours, we can better develop artificial systems in the laboratory to replace the use of animals, systems such as our multi-cell culture cancer model which we have used to help identify the metabolic communication between cancer-associated fibroblasts and the epithelial component of the cancer.

3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the lowest phylogenetic species that possess the necessary anatomical structures (a mammary gland) to conduct the study. In order to minimize harm done to the animals, we will mainly utilized an approach were-by we transplant tumour cells under the skin of normal or immune-deficient animals instead of using transgenic mice with oncogene activation, thus avoiding potentially more severe phenotypes.

PROJECT 12	**Ribosomal and metabolic stress responses in cancer**	
Key Words (max. 5 words)	Ribosome, metabolism, cancer, therapy, stress	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We are trying to understand how growth-promoting oncogenes give rise to cellular stresses and how cells respond to those stresses. We are particularly interested in two types of stresses: stresses associated with the ribosome (the protein production factory of the cell) and stresses associated with cell metabolism. We believe that these stresses might be widespread in cancer and might therefore provide clues about therapy in a wide variety of 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)?	We anticipate that our research will lead to new understanding in the processes that drive cancer development and will therefore create a knowledge base that will contribute to future cancer therapies. We are also trying directly to test novel types of cancer therapies based on our previous research and if they appear promising we will continue	

	to develop them towards clinical use.
What species and approximate numbers of animals do you expect to use over what period of time?	We anticipate that we will use 4,000 adult mice over a 5 year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most of the genetic changes that we make remain 'silent' until we perform our actual experiments. This allows us to make a genetic change (e.g. activate an oncogene) in a mouse and perform the experiment in the shortest possible time. From our previous experience of this approach, many of our experiments have almost no observable detrimental effect on the wellbeing of the animals. Some of our experiments are designed to address questions of tumour development and tumour therapy. For these experiments, we produce tumours in mice according to established protocols. The tumours are not allowed to develop beyond a strict size limit so that the mice experience minimal suffering. Guidelines for tumour size, as well as all other standard veterinary procedures, are laid down by the Biological Services department at the University. Furthermore, veterinary surgeons and experienced animal care staff are always available for advice and help since the welfare of the animals is of major concern to us. All mice are culled at the end of experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We study stresses and stress responses within cells during tumour development. These processes are highly sensitive to the environment in which cells find themselves. Thus, for relevance to cancer, we are obliged to study cells that are becoming cancerous in their normal tissue environment — i.e. within an animal. Where we can answer specific questions about these processes without using animals (for instance using cells in flasks) we do so.

	In fact, the majority of the work that we do is based on such systems and does not require animals. Usually a great deal of non-animal work gives us the information that we need to perform a limited number of animal experiments. These experiments can then reveal how these processes really work in a living animal and how they might be relevant in cancer.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We design our experiments so that the minimum number of mice are used to achieve a valid scientific result. There are several components that we incorporate to achieve this goal, including: • Statistical (power) calculations during experiment design to ensure that any data arising are scientifically valid — this prevents unnecessary repetition of experiments. • Pilot experiments with small numbers of mice so that experimental conditions can be optimised prior to larger experiments. • Special genetic techniques that allow us to breed less mice per experiment.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We have chosen mice to use as they are the animals that are most similar to humans that are readily amenable to genetic modification — this means that, for us, they are the best model to answer our questions about cancer development and therapy. The protocols are designed to generate as much information as possible from as few mice as possible with the least harm possible. Thus we make use of several advanced technologies that help us achieve this goal. In particular, we usually use 'silent' genetic changes that are only active for a short period during the experiment itself, minimising any undesirable effect of such changes. Anaesthetics are used for any procedures that would be expected to cause temporary discomfort. We are experienced in most of the protocols listed and know them to create minimal suffering. Where we intend to

	perform a protocol that we are less familiar with, we do so in consultation with the veterinary surgeons so that suffering can be minimised.

<table>
<tr><td>PROJECT 13</td><td>Signalling Pathways in Physiology and Oncogenesis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Genes, Cells, Signalling, Development, Cancer</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Cells are the building blocks of all animals and humans. They fulfil specific tasks within the organs in which they reside. Cells have to adapt to changes in their surrounding by communication with other cells, using chemical processes which the scientists call “signalling”. This is normally achieved by switching on or off certain protein molecules that reside on the cell surface. Information is passed into the cell by changes of signalling proteins until it is received in the cell nucleus which is the command centre for the cell. Within the nucleus, genes may be activated that have specific tasks linked with the event that triggered the signalling. For example, immune cells receive the signal that an infection has occurred and respond by instructing their genes to produce defence molecules such as antibodies. Signalling is also at the heart of how an egg develops into a</td></tr>
</table>

	complete organism, and how, in an adult organism, organs replenish themselves, for example in the gut or the skin. In disease, signalling often has become defective and wrong tasks are carried out by failing to activating the right set of genes. A particular case of faulty signalling is observed in cancer. This is because of defects in specific genes that send instructions for cells to grow limitlessly. The consequence of limitless growth is tumour formation. While scientists have identified a great number of genes that control tumour growth we expect to identify many more genes that would explain the growth of certain cancers of which we have little understanding at the moment. We also have insufficient knowledge about how the many genes that exist in the body interact with each other and how they fulfil specific tasks in different organs. However important inroads into the understanding of cell signalling have been made when scientists began to understand how specific genes are operating normally, for example during development. To do this, researchers would inactivate specific genes, for example by altering the DNA. They then ask how cells with altered genes behave, for example when they are instructed to grow, or when they are challenged with an adverse environment. While gene alterations have been very instructive for the understanding of cells using tools in the laboratory, their real importance (in development or cancer) only becomes apparent when they are studied in a live organism.
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)?	Biological research has found important roles of signalling molecules in development and cancer. Recent findings have also identified new components of certain signalling events. Some of these appear to be important in cancer development and require further scientific analysis. For this we will carry out experiments using laboratory tools but also mice. The aim is to assign specific roles for these new genes both in development and cancer. For example we will induce tumours in mice and determine whether

	changes in specific signalling genes are beneficial or detrimental to the tumour growth. Ultimately, our aim is to help in the further understanding of human biology and improving treatment of diseases such as cancer.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5 year period of this project we may use up to 4000 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?	For all the procedures proposed in this licence the limit has been set as moderate. We don't expect adverse effects during the vast majority of procedures. In cases where adverse effect occur, they are expected to be temporary and moderate and in any case, animals that show signs of adverse effects that are beyond moderate will be killed humanely. Tumour growth is not associated with pain, at least during the period in which we conduct our observations. For some procedures that involve surgery, we will administer pain killers and monitor closely. At the end of a procedure animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	For the vast majority of experiments we don't use animals, but instead cells grown in the laboratory. Their use enables us to answer most questions regarding specific scientific problems. However, no laboratory technique can entirely replace the use of animals for questions regarding development of a whole organism or the interactions between different cell types in cancer.
2. Reduction Explain how you will assure the use of minimum numbers of animals	As a rule, we use the smallest number of animals necessary to answer a specific scientific question. This will be backed up by many years of experience in choosing the right number of animals and by seeking advice on statistical methods to ensure the experiment will be conducted properly.
3. Refinement Explain the choice of species	Wherever possible we chose experiments that affect only the cell type that is under investigation,

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.	rather than the whole animal. This ensures that possible suffering is avoided or kept to a minimum.

<table>
<tr><td>PROJECT 14</td><td>Targeted mouse models for pre-clinical studies</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Genetically engineered mouse models, Cancer, Targeted therapeutics, Pre-clinical</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Regulatory use and routine production</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Preservation of species</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Higher education or training</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td><s>No</s></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The past decade has seen dramatic advances in cancer care, especially in better screening methods and earlier detection, and more effective therapies. This has led to increased survival rates both in adults as well as in in children and adolescents. In paediatric cancer, however, much of the progress has been restricted to leukaemia and lymphoma and some solid tumours. Progress has been more limited in paediatric brain tumours, neuroblastoma and rhabdomyosarcoma, which collectively represent a majority solid tumours seen in children and adolescents. Many high-risk forms of these diseases with particularly poor outcomes are associated with abnormalities of the MYC and MYCN genes. Even with very intensive therapies, the majority of these children and adolescents still die.

Moreover, where we have made progress in treating paediatric tumours, the improvement in outcome has come at a high cost. Two-thirds of</td></tr>
</table>

	children and adolescents who survive cancer face at least one chronic health condition. One third of survivors face a late-effect from treatment that is classified as severe or life-threatening. Late-effects of treatment can include heart damage, second cancers, lung damage, infertility, cognitive impairment, growth deficits, hearing loss, and more. There is therefore an urgent need for novel therapeutics for paediatric cancers, in particular, with very poor outcome as well as better therapies with fewer long-term toxicities for cancers that are treatable. Mutations in the *MYC* and *MYCN* genes are also implicated in many adult cancers, including aggressive and therapy-resistant prostate cancer. Thus, the objective of this project is to use genetically engineered mouse models (GEMMs) to develop novel "stratified" cancer treatments. We will continue our development of GEMMs of adult and paediatric solid tumours that overexpress cancer-causing oncogenes (with a focus on *MYC* and *MYCN*), in order to: 1. better understand the mechanisms by which oncogenes drive the formation of tumours, and 2. develop more safe and effective treatments for these deadly cancers. The GEMMs that we will use permit the oncogenes to switched on and off – an approach that allows for specific induction and withdrawal of oncogene expression. This enables us to better understand how oncogenes initiate tumours and ensure that new therapies are hitting the correct targets. We will also use immune-compromised mice that permit the growth of human tumours from implanted 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)?	There remains a need for new and improved mouse models for solid tumours, especially in paediatrics, this project will represent a significant advancement in the field that will permit analysis of tumours and new therapies in a realistic environment. In paediatric patients in particular, treatment of relapsed solid tumours is associated with severe side effects. Very few non-traditional or novel therapeutics have been implemented in paediatric cancer care, but the use of such agents is anticipated be associated with lowered toxicity. The development of regulatable, cancer GEMMs that

	are sensitive to oncogene withdrawal will help determine the potential efficacy of targeted therapeutics and how they might be implemented clinically. Success in this work will contribute novel therapeutics for the treatment of incurable paediatric as well as adult cancers and will help to unravel the mechanisms by which dysfunction of oncogenes such as MYC and MYCN drives tumourigenesis in these conditions.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use the house mouse (*Mus musculus*). The mice that we will use will be genetically altered to predispose them to various cancers. We will also use immune-compromised mice. The approximate numbers of mice we expect to use during the 5-year project are between 30.000 and 35,000.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Although we do everything possible to minimise adverse effects, these can occur. Possible symptoms include loss of condition, weight loss and effects on organs, e.g. skin or liver. Mice are checked regularly for signs of ill health and specific score sheets provide objective records and defined endpoints.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are no suitable ways to properly model these tumours *in vitro* using cancer cell lines. In order to study tumours that form spontaneously, within a native host environment and at the appropriate time during development, GEMMs are the only practical tools available to us. GEMMs also allow us to model how the presence of the same mutations found in human cancers affect tumour development and response to therapies. To study the effect of novel treatments on human tumours, we also use immune-compromised mice that allow the growth of cells taken from patients. Together, these models are complementary and predict quite well the activity of drugs in the clinic.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Most drug development utilises rodents, so we have a considerable amount of information on existing drugs for comparison with novel therapies. Before any new agent is administered we first perform extensive *in vitro* laboratory experiments to establish the concentrations and exposure time required. Then we test that any novel agents are tolerated in animals.

	Mouse numbers are calculated to minimise usage while allowing robust and statistically significant results.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We operate within a very tightly regulated, clean and well administered facility that has an excellent track record for animal care and safety. In all cases we use appropriate anaesthetics and procedures to avoid pain, suffering or lasting harm to the animals. When we have to kill them we do so by approved procedures. We have also implemented a real-time, networked database to monitor colony dynamics and events, so that we are always aware of issues with our animals and can respond to them quickly. Additionally, we are refining and replacing our current models with more sophisticated ones that incorporate non-invasive tumour imaging to enable early tumour detection. This will reduce animal numbers, shorten our experiments, and prevent large and potentially debilitating tumours from escaping detection. In this way we hope that efficient and compassionate use of our animals will help us to make a lasting impact on the treatment of these deadly cancers.

PROJECT 15	**Targeted Therapy for Cancer**
Key Words (max. 5 words)	Cancer, Gene therapy, Radiation, Radiosensitiser, Virus therapy

- Summarise your project (1-2 sentences)

This project aims to use new therapies to improve treatment outcomes for cancer. A significant component of the work will involve using viruses either to deliver genes to tumour (or normal) tissues or as cancer killing agents in their own right. In addition, we will also evaluate classes of new drugs that are able to make radiation more effective in killing cancer cells.

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

We are conducting this work to improve treatment outcomes of patients with cancer. Specifically, this work aims to improve the effect of radiotherapy and/or chemotherapy – through using virus therapies or drugs that increase the cancer-killing ability of radiation or by protecting normal tissues from damage caused by radiation. This work is needed because radiation and/or chemotherapy are frequently ineffective at controlling cancer and new developments are urgently needed.

- Outline the general project plan.

Our overarching goal is to improve treatment outcomes in cancer. There are 3 specific areas in which we will work. First, we will test viruses, either as vehicles for delivery of therapeutic genes or as therapies in their own right, in combination with radiotherapy and/or drug treatment. This work builds on a number of established and published models in which we have used viruses to deliver proteins that can activate innocuous prodrugs or drive uptake of therapeutic radioisotopes. Alternatively, we have used viruses that are selectively capable of replicating in tumour cells to sensitise to radiation and/or drug treatment. This work has progressed to the clinic and the ongoing studies in this programme aim to refine our approaches to improve anti-cancer effects. Second, we will expand our experience in the use of surgical techniques to improve our ability to administer gene or virus therapy. These studies will be based on free tissue flaps or isolated limb perfusion circuits to restrict the delivery of a virus to a specific territory where it can have a localised effect. The third approach aims to develop new drugs (so-celled radiosensitisers) that increase the effect of radiation on cancer cells without affecting the response of normal tissues.

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

Most of the animals included in this work will receive injections of human (or mouse/rat) cancer cells in order to cause them to develop cancer. These injections and the resulting cancer masses may cause discomfort. Tumour bearing animals will

receive treatment with radiation or drug therapy. The former will be administered under general anaesthesia and the latter by intratumoural, intraperitoneal or intravenous injection or by gavage. Again, these procedures may cause some discomfort. In some instances, rats may undergo surgical procedures to generate free tissue flaps for gene therapy or isolated limb perfusion for tumour therapy. All procedures will be performed under general anaesthesia. In the post-operative period, the animals may suffer some discomfort and local inflammation at the surgical site.

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

These experiments will allow us to continue to translate our laboratory work in to high quality clinical trials in patients with cancer. We anticipate generating new treatment approaches combining radiation and/or chemotherapy with virus therapy or targeted drugs that increase the benefits of radiotherapy.

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

We anticipate using no more than 3000 mice and no more than 1500 rats over 5 years. The mice will mainly be immunodeficient animals to enable us to study the activity of new treatments against human tumour cell lines, but some experiments will also be conducted in immunocompetent animals in order to evaluate the effects of the immune system. Rats will be used in the surgical studies of free flap transfer and isolated limb perfusion. These studies are not possible in mice because their blood vessels are of insufficient diameter to allow effective microsurgery. The number of animals used will be minimised by conducting exhaustive in vitro analyses before proceeding to test our data in in vivo studies. We will also estimate the size of the likely therapeutic effect in order to perform power calculations that will avoid designing experiments with group sizes that are too large.

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

In all aspects of our work, we will conduct extensive in vitro analysis before conducting in vivo studies. This will include definition of the most appropriate cell lines to use for in vivo studies. This approach has limited the use of inappropriate tumour models and has reduced our total numbers. In addition, in vitro experiments have been used to refine aspects of treatment scheduling with a view to limiting the use of animals in large exploratory in vivo studies. For the surgical models of free flap transfer and isolated limb perfusion, there is no alternative to in vivo experiments. However, whenever possible, we refine our experimental designs by initial in vitro analyses before resorting to in vivo testing.

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

Our work will be based on humane treatment of animals at all times. Indeed, I have employed a full-time qualified Animal Technician to ensure the highest standards are maintained in our work. For all procedures that may cause discomfort, the period in which the animal may experience pain is kept to a bare minimum and where necessary anaesthesia and analgesia are employed. Following procedures, animals are closely observed and those that show persisting signs of distress are treated appropriately or euthanized (depending on the severity and recoverability of the condition).

<table>
<tr><td>PROJECT 16</td><td colspan="3">Temporal analysis of cancer evolution in vivo</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Cancer, Biomarker, Therapy</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3"><u>Objectives:</u> Over 30 years of molecular biological research has proven beyond a shadow of doubt that specific genetic mutations can combine to cause cancer. Moreover, from the analysis of such mutations in simple tissue culture-based systems we have an enormous volume of information on how these mutations alter cells and their behaviours in ways that benefit cancer. However, rationally designed drugs based on this volume of data have, in the vast majority of cases, failed to deliver meaningful extensions of human lifespan and/or quality of life. In part, this is a consequence of inadequate molecular tools and a heavy reliance on surrogate systems that fail to fully recapitulate how a developing tumour interacts with its natural environment. Using a highly refined set of genetically engineered mice, combined with a range of highly sensitive whole body imaging modalities, we plan to study the entire process of</td></tr>
</table>

	cancer evolution, from the very first day a cell is exposed to a cancer causing mutation. This will enable us to learn more about the earlier stages of cancer development about which very little is really known and to identify new therapeutic opportunities that arise during cancer progression.
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)?	Predicted Benefit: In some instances we will develop entirely new models of human cancers, while in others we will refine existing models to make them more informative and to better recapitulate the human disease experience (eg. sporadic activation of mutations in a small number of adult cell). The use of these mice will significantly add to the knowledge base of a broad spectrum of cancer researchers and moreover identify new biomarkers for early detection as well as novel candidate therapeutic targets. As such, we expect to see additional benefits eventually trickling through to cancer patients in the years to come.
What species and approximate numbers of animals do you expect to use over what period of time?	Genetically Engineered Mice: Over a five year period we estimate generating up to 20,000 mice through breeding, with some 2,500 being used for experimentation and analysis
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	In the vast majority of instances, our mice will be euthanized long before they endure any discomfort or exhibit any signs of distress arising from the developing tumours. The majority of mice will endure no more procedural discomfort than arises from a small needle injection. A small minority will undergo keyhole surgery under anaesthesia and will receive analgesia both pre- and post-operation. A very small minority may experience discomfort arising from candidate therapeutic agents, which will always be used at sub lethal doses. Severity levels will not exceed moderate. All mice will be euthanized humanely via a schedule 1 method.
Application of the 3Rs	
1. Replacement	Cancer is an incredibly complex disease involving

State why you need to use animals and why you cannot use non-animal alternatives	many normal cell types as well as the cancer cells themselves and host of additional interactions between the cancer cells and their natural environment. Only from studying cancer evolution in its natural setting can we fully learn about the holistic process of tumour development. Moreover, proper pre-clinical evaluation of potential therapeutics demands the development of and testing in models that accurately and faithfully recapitulate human cancers. However, we will certainly use cell culture based systems to pre-validate potential therapeutics thoroughly before progressing to in vivo analysis.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will ensure that a minimum number of animals are used by a) refining our breeding schemes to increase the frequency of experimentally useful genotypes amongst progeny; b) consulting with institutional statisticians to ensure experiments are adequately powered and c) performing non-invasive longitudinal analysis of individual mice (eg. via ultrasound or PET/CT imaging of tumours before and after intervention).
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Only in mice do we have an extensive library of engineered mutations representative of those arising in human cancers. These genetically engineered mouse models (GEMMs) have a proven track record in faithfully recapitulating human cancers at every level. Moreover, only in GEMMs is it possible to experimentally follow the entire course of tumour development from inception. Our protocols aim to give us ultimate control over the timing of tumor initiation, thereby allowing us to glean entirely new information about how cancers develop and how they interact with their natural environment. Our methods are geared precisely to intervene well before the animals experience any level of discomfort or distress arising from their nascent cancers and to minimize discomfort during experimental procedures.

<table>
<tr><td>PROJECT 17</td><td>Manipulating cellular and microenvironment crosstalk in chronic inflammation and cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Lung cancer, inflammation, microenvironment,</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>We aim to investigate key genetic and molecular signalling pathways involved in chronic respiratory disorders with an inflammatory component; including lung cancer, lung fibrosis and mesothelioma.

The clinical course of these conditions is characterised by progressive decline and an extremely poor response to current treatment options. There is an enormous need for good research of a basic and translational nature into these devastating conditions.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>We aim to improve scientific understanding of how inflammation can impact on the process of carcinogenesis using the above disease models.

We hope this will lead to recognition of key molecular structures, which can then be targeted</td></tr>
</table>

	with curative intent. An important part of this group's ongoing work is to construct a novel in-vitro 3D tumour model, which will allow us to manipulate elements of the tumour environment and assess how this impacts on tumour growth and treatment. We would expect this to drive down our in vivo animal requirements.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice. Approx numbers of 2600 mice required over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Adverse effects are largely related to tumour growth, particularly in subcutaneous tumour implantation models upon reaching a certain size. We will monitor mice that receive tumour cells very carefully and if there are any signs of pain or distress (before the tumour reaches pre-defined size limits) the animals will be humanely killed. Within this body of work there are no protocols more severe than 'moderate' level and animals will be humanely killed by Schedule 1 method after experimentation.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although we utilise in vivo techniques as much as possible, the field of inflammatory microenvironmental investigation requires in vivo models to adequately recapitulate live body variables such as tumour oxygen levels, blood supply, and the stiffness of surrounding supportive tissue.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments have been designed with input from Statistics department in order to ensure minimal animal numbers in experiments whilst ensuring the experiments are adequately powered. Non invasive imaging techniques will also drive down numbers as there will be less of a need for routine sacrifice to assess tumour progression.

3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the species of lowest neurophysiological sensitivity that provide the necessary size to allow us to initiate, image and monitor our cancer models with adequate resolution. We will try to reduce suffering as much as possible during all invasive procedures. Inhalational anaesthesia will be used to minimise transient pain and distress, where possible, during tumour induction, sampling, measurement and combined with imaging procedures. Animals will also have full recovery between periods of anaesthesia, rehydration during long imaging sessions, monitoring of respiration/cardiac function and maintenance of body temperature during imaging. Welfare score sheets and frequent monitoring will be used to assess progression of tumour burden, animal health and behaviour so humane end-points are reached well before onset of clinical adverse effects.

PROJECT 18	**Gene function in tumorigenesis**	
Key Words (max. 5 words)	Cancer, colorectal cancer, drug targets, targeted drug delivery, diagnosis	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Cancer is responsible for more than one in four deaths in the UK and in 2010, there were around 157,250 deaths from cancer. Four cancers - lung, bowel, breast and prostate - account for almost half of all cancer deaths in the UK. Colorectal cancer is the second commonest cause of cancer related death in the UK with 16000 deaths annually. Incidence is also increasing highlighting the need for improvements in colorectal cancer prevention, diagnosis and treatment, particularly in the context of an ageing population. To achieve improvements in cancer diagnosis and treatment, a more detailed understanding of the molecular mechanisms that lead to cancer is required. This will lead to the identification of novel targets for diagnosis and prognosis as well as drug and therapeutic development.	
What are the potential benefits likely to derive from this project (how science could be	Pre-clinical investigations and translation will, in time, facilitate a more personalised approach to cancer treatment where the right treatment can be given to	

advanced or humans or animals could benefit from the project)?	the right patient at the right time. This will improve outcome and quality-of-life for patients.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, up to 3300 in 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Animals that are genetically engineered to develop spontaneous tumours or experimental models with implanted human cancer cells will develop tumours. Tumour growth and development will be closely monitored to ensure that adverse effects are minimised and humane endpoints are ob served as well as the moderate severity limit. All animals will be humanely killed at the end of the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Cancer is a complex disease involving all aspects of human physiology including different cell types and organs, cardiovascular system, nervous system and immune responses. In order to fully understand the molecular mechanisms that drive this complex disease it is essential at some point to study gene function and tumorigenesis as well as novel therapeutics in the context of the whole living animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	New investigations will involve pilot study groups to determine any effect and also provide data for statistical analysis to determine sample size in further studies. Non- invasive imaging will be used where possible to reduce the number of animals used by enabling longitudinal studies and therefore reducing the need for large cohort 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 measur4es you will take to minimise welfare costs	The range of mouse models available for cancer research and the ethical framework in place makes the mouse the model of choice for this work. The availability of genetically altered mouse models means that models are available where tumours arise spontaneously in specific organs and therefore reproduce human cancer phenotypes. We will use the most appropriate cancer models available to address specific objectives.

(harms) to the animals.	Non invasive imaging will be used where possible and will ensure that humane endpoints are adhered to as more accurate detection and analysis of tumour growth informs decision making.

<table>
<tr><td>PROJECT 19</td><td colspan="3">Biochemical investigations of the heart</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Heart failure, metabolism, mitochondria, contractile dysfunction</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">The overall aim of this research is increase our understanding of the cellular mechanisms underlying the abnormalities in cardiac muscle function and energy metabolism that occur in the failing heart, using in vivo non-invasive and translational techniques.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">The information arising from this project may help to identify early diagnostic and prognostic markers of cardiac hypertrophy and new targets for the development of novel therapies for heart failure, aimed at optimizing energy provision, metabolism and preserving mitochondrial function.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td colspan="3">Primarily the aim is to use adult rats with a maximal annual average of 150 animals</td></tr>
</table>

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?	Although some of the procedures are severe in their induction, the animals make a good recovery and thus would be classified as moderate. Using non-invasive techniques will allow repeated monitoring of the animals over a maximum of 6 months. The animals will be sacrificed via a non-schedule 1 procedure 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	Alternative strategies to the use of animal models have been carefully considered but there are no non-sentient alternatives to answer many of the important medical, biochemical and physiological questions in the field of heart failure. A mouse atrial tumour cell line, HL-1 cell line, does exist which has been shown to mimic many of the metabolic properties of the cardiomyocyte and this cell line will be used in place of animal tissue where possible.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The minimum number of animals for statistical significance will be used based on past experience. Numbers will be reduced by the use of non-invasive techniques, such as positron emission tomography and magnetic resonance imaging (MRI & MRS) to monitor cardiac metabolism and function non-invasively over time rather than using many animals at each time point to monitor ongoing changes.
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 aim of this project is to use non-invasive techniques wherever possible and seek regular advice and updates on techniques, such as anaesthesia and surgery, to ensure best practice. Discomfort and injury to animals will be limited to that which is unavoidable in the conduct of scientifically valuable research and analgesic, anaesthetic, and/or tranquillising drugs will continue to be used where appropriate to minimize pain and/or distress to animals

PROJECT 20	**Analysis of cancer gene phenotypes**	
Key Words (max. 5 words)	Oncogene, tumour suppressor gene, tumour formation	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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)	Some of the genes involved in the development of cancer in humans are known, but there are many other genes that when altered are likely to play a role in cancer formation and progression. The objectives of this project are to: (a) Identify genes that drive tumour formation. (b) Investigate how these genes contribute to tumour development and determine the normal functions of these genes.	

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Cancer is due to abnormal and uncontrolled cell proliferation that is caused by both genetic and environmental factors. Although much work has been done to improve the understanding of the molecular events that occur during tumour formation, many cancers remain poorly understood and some are incurable. This project proposes the use of mice to better understand the genes involved and their molecular mechanisms in tumour formation and progression in humans. Mice represent the ideal model for this study, based on the similarities between humans and mice in terms of anatomical, physiological, pathological and genetic features, together with the ability to make specific changes to mouse genes. This project intends to generate and characterise mice that have mutations in known genes or newly identified cancer genes or combinations of genes that increase susceptibility to tumour formation. This will advance scientific understanding of the processes of formation and progression of cancer. The work will identify and characterise new cancer genes, which may open up new possible avenues for the development of anticancer drugs that target these genes and their pathways.
What species and approximate numbers of animals do you expect to use over what period of time?	Genetically altered mouse studies allow changes to be made to specific genes in particular tissues, with study of all stages of development of cancer, including the interactions between genes, cells, tissues, and between tumour cells and the surrounding microenvironment. Importantly, such mouse studies can be used for preclinical trials of potential anti-cancer therapies. It is predicted that approximately 8,000 mice will be used over the five years of the programme.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Plan of work: (a) To genetidally modify selected genes to study how they contribute to tumour development and progression as well as how they participate in normal processes. (b) To mutate mouse genes in order to determine which gene mutations contribute to tumour development. (c) To use exposure to potentially tumour causing agents to understand how the environment may contribute to or modulate tumour development. This work will use genetically altered mlce produced in other laboratories. All of the animals will be housed

	in a modern animal care facility and will be monitored daily for signs of illness due to tumour formation or other causes and thus the expected adverse effects due to tumours will be kept to a minimum, mostly at the mild level of severity (although occasionally moderate). At the end of the studies the mice will be humanely killed and dissected to analyse tumour formation and progression. The work will be performed in accordance with the principles in the Guidelines for the Welfare and Use of Animals in Cancer Research: British Journal of Cancer (2010) 102, 1555-1577.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The cellular interactions, both cell-cell interactions and cell-environment interactions, involved in cancer formation can be studied in mouse mod&s, whereas they can't be studied in the same way in ex *vivo* human cancer samples or using *in vitro* studies of cancer cell lines. Where possible, we will use *in vitro* studies of cancer cell lines instead of animal studies for some functional studies of gene effects not involving cellular interactions with other different cell types or the environment.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The protocols and number of animals used in these experiments have been statistically optimized to ensure that the minimum number of animals is used and that any adverse effects of the genetic alterations are kept to a minimum. Where possible, mice with existing abnormal genes will be imported. Where possible, we plan to use both *in vitro* cell line studies for tunctional analyses of gene effects and small pilot *in vivo* experiments prior to determining the final experimental design that involves the minimum number of animals. We propose to generate and utilise several different genetically defined mouse strains, which will all bear genetic changes known or suspected to be associated with tumour formation, and are relatively well characterised in terms of their predisposition to tumour formation, so we can utilise these data to design experiments with the minimal animal usage required to give significance.
3. Refinement	Mice are well defined genetically and their genes can

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.	be readily altered. There are in existence already, several mutant strains of mice including those with genetic alterations in cancer genes relevant to this programme of work. Hence, mice are a particularly good choice for modelling human cancer and for investigating the basic biological mechanisms involved. We already have much experience in looking after mice that develop tumours, in particular Looking for the early signs of tumour formation. Our emphasis will be focussed on sound experimental design to test our hypotheses, based on experience from our own previous work, use of organ-specific or tissue-specific gene alterations, and appropriate use of statistical tests of significance. Harm will be minimized by careful daily observation of the mice for signs of illness, particularly the early signs of tumour development, with use of well designed protocols based on previous experience. We aim to gain the information we need before there is a significant negative impact on the animals welfare.

PROJECT 21	**Genetic determinants of cancer metastasis**	
Key Words (max. 5 words)	cancer, metastasis	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The spread of tumours to distant organs in a process termed metastasis is one of the most devastating aspects of cancer, being the cause of ~90% of cancer- related deaths. The vast majority of metastatic cancers are refractory to treatment and are therefore incurable. Thus, there is a pressing clinical need for further research on the molecular basis of metastatic cancer. The goal of this project is to understand genetic factors that are required for metastatic cancer. Such knowledge is essential for the development of rational treatment strategies for the metastatic disease.	

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The expected benefit from this work is a better understanding of the molecular mechanism that drive cancer metastasis. This knowledge will serve as a basis for the development of rational therapeutic strategies to combat metastatic cancer. Thus, the potential secondary benefits of this work will go beyond basic cancer research, possibly leading to the identification of (i) new molecular targets for metastatic cancer and (ii) novel predictive and/or prognostic biomarkers for metastatic cancer.
What species and approximate numbers of animals do you expect to use over what period of time?	Our work focuses exclusively on mouse models. We expect to use about 2000 animals over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Tumour induction itself will in most cases have no significant impact on animals' general well being. Short- term mild discomfort may be possible directly at the injection site but animals are expected to recover quickly. In some cases, such as where surgical procedures are used, the mice will receive analgesics in order to reduce the injection-related adverse effects. In rare occasions tumour induction may result in sudden death without preceding signs. After tumour induction, animals will be observed closely for any evidence of tumour growth by careful palpation, inspection of the injection site and whole animal imaging. Discomfort resulting in moderate clinical signs such as hunched posture and inactivity or respiratory distress will result in individual animals being killed and on occasion the termination of the experiment. For other procedures such as pharmacological treatment most animals will show no more than mild clinical signs. Some may show moderate signs such as hunched posture, loss of body weight, lack of grooming, which will require culling. At the end of experiments, all animals will be killed.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	A large proportion of our research programme employs cell culture models and analysis of publically available human data sets, which will be used to investigate our hypothesis. However, metastasis is a complex process that involves numerous normal cell types and tissue structures that cannot be reconstructed in cell culture models. We will therefore utilize animal models in order to validate our findings in a more physiological setting and in the context of preclinical studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Careful planning and collaboration with experienced statisticians will help us use the smallest number of animals that will ensure robust results. Once the use of minimum numbers experimental end points are reached, cancer from of animals affected animals will be harvested to facilitate continuing, complementary ex vivo/in vitro studies. Pilot studies on small cohorts of animals will reduce the number of animals used. Imaging based techniques such as bioluminescence will allow further reduction in animal usage.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mouse models have been chosen as they represent the least sentient species able to generate meaningful data, i.e.that is likely to be directly applicable to the human disease. Animal suffering is minimised by the use of appropriate anaesthetic and analgesia. Where clinical signs are seen animals will be culled as soon as possible and before they are likely to develop signs of pain or distress that would exceed a moderate severity limit.

<table>
<tr><td>PROJECT 22</td><td>Bispecific antibodies for use in cancer therapies</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cancer, immunotherapy, bispecific antibodies</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>Five</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>1. A method to validate new combinations of targets which have already proven successful when used singly in cancer models.

2. A method for improving current combinations of antibody therapies (doses each individual antibody separately) through the use of administration of a single bispecific antibody.

3. An understanding of the cellular basis for inhibiting or enhancing the immune response in relation to cancer therapies.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the</td><td>1. Novel cancer immunotherapy using bispecific antibodies.

2. Increased efficacy over current monospecific therapeutic antibodies.</td></tr>
</table>

project)?	3. Directly translatable into human therapies
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse: 6,800 over 5 year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The expected level of severity of the overall application would be moderate. With regards to individual expected adverse effects of specific protocols, such as proposed models of HER2+ breast cancer, phenotypic characteristics will be closely monitored and addressed immediately as they occur. In all cases of tumour study, anaemia, weight loss, ascites, pronounced lethargy and decreased interaction may be observed and will be addressed immediately as they occur to minimise any and all suffering in the mice. At the end of planned procedures, mice will be humanely killed by competent and trained persons working under the project licence.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The biological and health-related questions of cancer immunotherapy and the maintenance of tissues and organs cannot in the present state of knowledge be addressed by in vitro methodology. Considerable work is undertaken to fully characterise bispecific antibodies in vitro, but validation in a complex competent intact immune system setting is essential for future translation into improving human health.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Every opportunity will be taken to minimise the number of animals used for each experiment whilst still maintaining the statistical relevance of the subsequent data. Typically, the desired observed effect will be large, and the need to keep sample size to a minimum will be observed as long as results will continue to be statistically significant. To maximise information, multiple body sites will be examined from each animal and multiple analysis types will be conducted on each sample, where possible. Breeding of animals will be carefully

	monitored to ensure no excessive breeding occurs.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We will use unique, genetically modified mouse strains when possible, that refine our capacity to design and conduct precise experiments that focus on the cells that are most important and relevant to human biology and diseases. In addition, mice will be kept under regular monitoring and observation to maintain good general welfare and minimise any risk of undue suffering.

<table>
<tr><td>PROJECT 23</td><td>Diagnosis and Therapy of Gastrointestinal Cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cancer, Abdomen, Optical Imaging</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>24 months</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>This is a proof of principle study to investigate the application of very small gold particles (invisible to the naked eye) that can heat up after being irradiated by near infrared light (outside light’s visible spectrum). Heating is an established means of destroying cancer cells. It is expected that these very small gold particles can be delivered to the site of tumour through the blood stream where there are leaky blood vessels or directly into the tumour.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>By applying near infrared directly to the tumour site that has retained the gold particles, the heating that occurs will hopefully destroy cancer cells. This would be a foundation to continue developing the model further towards the application of photothermal therapy in humans</td></tr>
</table>

What species and approximate numbers of animals do you expect to use over what period of time?	Mice – 60 - over a period of 24 months.
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 potential adverse effects are infection of the tumour implantation or biopsy site (<0.5%), pain (from the treatment) which will be managed with antibiotics where indicated and painkillers such as anti-inflammatories and morphine-derivatives. All animals will be monitored assiduously for changes in physical appearance, behaviour and social interaction and are to be killed by an established method if there are unexpected or untreatable deviations. All animals will be ultimately killed if the tumour grows to larger than 1.2 cm. Level of severity = moderate. Should there be any unexpected gross weight loss or tumour ulceration, this would also be factors for early termination.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Delivery of the gold particles to the tumour site requires circulating blood and an anatomical and physiological system in order for the proposed mechanism of cellular destruction to function and, ultimately, be useable in humans. Research has demonstrated that gold particle technology is effective in cells and human *ex vivo* tissues. This is the beginning of the work to test the efficacy of this system in live animals and, eventually, humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	This is a proof of concept study. Previous *in vitro* and ex vivo work suggests that, if the experiments perform as predicted, 50 mice will provide sufficient data to show that this treatment is likely to be effective. To further limit the use of animals, we have minimised number of control animals required arm of the study to 10 animals, which is sufficient to provide comparisons for two experimental scenarios.
3. Refinement	Mice have been chosen for this study as the

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.	species with the lowest capacity to experience pain, suffering, distress or lasting harm but with a relevant physiology to test the principles of gold-particle tumour treatment. The use of subcutaneous sites for the investigation of tumours produced by gut cells minimises the invasiveness and pain associated with procedures for monitoring growth and response to treatment. Where possible non-invasive procedures will be used to treat and monitor tumours. Tumour size and the health of mice will be regularly monitored and action taken to alleviate pain and suffering where required and in accordance with the principles described in the National Cancer Research Institute guidelines. All published studies regarding the presence of GNP in the circulation suggests that there is no pain, suffering, distress or lasting harm to be derived from clinically relevant solutions of *in vivo* GNP administration. Re-using animals that only have a minor procedure such as a small biopsy of tumour sites would also reflect a refinement of procedure.

<table>
<tr><td>PROJECT 24</td><td>Action of and intervention in control pathways in cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Kinase, cancer, drugs</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Cancer is a public health burden on a global scale. Worldwide 14 million diagnoses and over 8 million deaths from cancer were recorded in 2012. Within the UK one in three people will be diagnosed with cancer at some point in their lives. This substantial unmet clinical need continues to drive the identification of opportunities for the development of new drugs. Increasingly in the 21st century efforts directed at new treatments in human disease are so-called targeted therapeutics – not simply identified as drugs able to modify symptoms but rather drugs directed at targets that are in some way defined as causative in the disease. This is well evidenced in cancer with the current use and ongoing development of new targeted therapeutics.

Amongst the targets that have been successfully exploited in cancer are a class of cellular regulators, collectively referred to as protein</td></tr>
</table>

	kinases. These proteins are disproportionately mutated in human cancer giving evidence for their roles as drivers of disease. Amongst this class of protein kinases is a subfamily referred to as PKC family that are themselves implicated in certain cancer-associated events/properties. Whether individual PKC family members are valid targets and if so in what cancer context might they be targeted is not resolved. The three family members/groups for which this programme intends to furnish this critical information are: PKC, PKCand PKN1-3. There are compelling reasons to seek validation of these candidate targets based on a combination of tumour analysis (PKCand PKN1-3) and ex vivo laboratory experimentation (all). In fact for PKC□/□ the weight of proof from tumour samples is such that these are considered validated targets, indeed we have developed drugs that can target them and the work here is very much part of the pre-clinical work up of these agents to determine where and when PKCinterventions may best work. In the case of PKC, we have developed a deep understanding of a dependency that emerges in ex vivo cancer models that simply does not exist in normal physiology. We need now to validate this behaviour in a whole animal setting before seeking drugs we could then take into a cancer trial. Mice have the same spectrum of PKC family members as humans and we have established a genetically engineered mouse with no PKC□. So we are now in a position to test its requirement, using cancers induced in a WT or PKC□ absent setting. For the PKN subfamily there is both patient tumour based data and ex vivo laboratory data to indicate roles in certain tumour types (including prostate and breast cancers). Here again we have developed genetically engineered PKN ablated mouse strains that will now enable us to assess their importance or not in cancer models in vivo. In this case we are seeking to assess roles using prostate and breast

	cancer models.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The expected benefits from the project are of two types. Firstly, we are progressing PKC□□□□targeted drugs into the clinic and the outputs of the project here will provide evidence on what aspect(s) of tumour development and progression (spread of disease to secondary sites) are influenced by PKC□□□□inhibition. Associated with this we will also test markers indicative of drug action i.e. target inhibition. This output is important for the clinical trial which will need to provide evidence on drug action/efficacy for the target. Secondly, for the other two objectives, the programme will provide evidence that one or both are valid targets. This is critical to the activation of drug development activities where the longer-term output would be early phase trials testing their potential value in treating one or more cancer types.
What species and approximate numbers of animals do you expect to use over what period of time?	We will pursue these studies in mice where the full repertoire of mammalian PKC family members are expressed – in lower eukaryotic model organisms there is a far less complex collection of PKCs. We anticipate requiring up to 7,500 animals over the entire 5 year period to cover the breeding programme for the specific genotypes to be tested in the cancer (prone) models.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The programme involves testing tumour formation in the context of manipulating potential drug targets. Hence the central adverse effect anticipated is the tumour load itself. This will be limited to moderate severity allowing for growth of the primary tumour to a point where we can address the problem of disease spread to secondary sites. For all of these studies we expect that the manipulation of the target (ablation or inhibition) will tend to improve animal welfare by reducing tumour load and/or tumour spread. Any animal displaying unexpected harmful phenotypes will be humanely killed. All animals will be killed at the end of each experiment.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Over the last two decades we have carried out extensive ex vivo studies on the functions of this family of candidate targets and we continue to do so. However in converting this knowledge from the promise of a potentially useful medical intervention into an experimentally validated target we need to test behaviours in the much more complex setting of a model organism. Because there is no direct conservation of these targets in simple model organisms such as the fruit fly, we need to turn to a mammalian model organism and the mouse is the one that fulfils the requirements of the programme.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have consulted experts in the use of engineered cancer prone models that we have planned to use. This has provided clarity on the frequency and timing of events that enables us to design each experiment with the minimum number of animals based on the predicted markers of events. With regard to the human tumour cell samples propagated in the mouse models, we have extensive prior experience with such models and again for individual tumour cell samples, we have consulted those with experience of their use to determine timing of events and frequency as well as optimum handling to provide consistent minimally variable results. All of our studies will be designed to provide statistically informative answers. Again, we have consulted on the biostatistics and will also employ national on-line design resources to support this work.
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	The mouse models provide the simplest model organism in which the human candidate target genes under study are present and hence the simplest which can be employed to test the validity of these targets in an experimental organismal setting (as opposed to a simple tissue culture context). In testing these candidate targets in

minimise welfare costs (harms) to the animals.	tumour models we will employ good husbandry practices and regular monitoring of all animals. We will increase monitoring immediately following any acute procedures. We will employ humane endpoints to minimize suffering especially in the case of unexpected, additional suffering. Where continuous monitoring, non-invasive data collection methods can be used, we will seek to use them implementing the earliest possible humane endpoint without compromising the validity of the experiment. We will balance the possible distress of such procedures (anaesthetics for imaging) against the benefits. With respect to the skin tumour promotion studies, we will give analgesia to animals where this does not invalidate the experiment.

<table>
<tr><td>PROJECT 25</td><td colspan="3">Investigation of RAS oncogene mutant cancers</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Lung cancer, therapy, early detection</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td></td></tr>
<tr><td>Preservation of species</td><td></td><td></td></tr>
<tr><td>Higher education or training</td><td></td><td></td></tr>
<tr><td>Forensic enquiries</td><td></td><td></td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td></td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">About a quarter of cancers are caused by mutations in a group of interconnecting growth regulatory molecules called the Ras pathway. So far attempts at blocking the function of the Ras pathway have been unsuccessful. There is therefore a need to develop new treatments for cancer patients where this pathway is important, in particular major killers such as lung cancer, colon cancer and pancreatic cancer. Work carried out in this laboratory in the past has aimed to identify novel ways of killing cancer cells with mutations causing overactive Ras pathway signaling. This was initially performed on cultured cells and then followed up in mice with predisposition to lung cancer or pancreatic cancer. We have found a number of new combinations of existing drugs that are able to cause major regressions of cancers in these mice, several of which are being taken forward into clinical testing in human patients.</td></tr>
</table>

	However, while this is very encouraging, we also know that tumours in these animals are not completely eliminated and will regrow after therapy is discontinued. We wish to continue these studies to address how we can turn short-term tumour regressions into long-term cures. In order to do this we wish to investigate the interaction of the immune system with tumour cells as they die in response to our new drug combination therapies. We plan to explore what parts of the immune system recognise the dying tumour cells and what is preventing the immune system from then fully rejecting the tumour. We will test whether precision targeting of some of the brakes on the immune system using so called “immune checkpoint inhibitors” might be able to work together with the drug combinations we have already tested to cause complete tumour destruction. A second aim of this project lies in the field of early diagnosis. In most common solid tumours, including those where Ras pathway mutations are frequent, early surgical intervention is by far the most effective therapeutic approach available at present. The most important limitation to the use of curative surgery is the fact that patients very often present with cancers that have already spread beyond their primary site, leading to metastases at multiple secondary sites that cannot all be removed surgically. Improved ability to detect cancers at a very early stage would allow greater usage of surgical intervention and may hold out the promise of greatly enhanced cure rates. Some of the most exciting developments in the area of early diagnosis of cancer in recent years have come from the ability to identify tumour derived DNA in circulating blood, known as circulating free tumour DNA. However, the mechanisms involved in release of DNA from tumour cells into the blood are poorly understood. In this project we plan to use tumour prone mice as a model system to explore the biology of circulating free tumour DNA and in particular to address whether there are ways in which its release and detection could be improved for the purpose of

	early detection of common cancers through simple blood tests.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The potential benefits of this work lie both in advancing basic scientific understanding of cancer as a disease, and, perhaps more importantly, in providing insights into new clinical strategies for the therapy of common cancers and also for its early detection. In the work on therapeutic drug combinations and promoting the ability of the immune system to work together with these to eliminate tumours, we hope to provide clear rationales for the design of new clinical trials in cancer patients that optimally combine the very latest targeted drug combinations with immunomodulatory drugs. For the work on early detection, we hope to understand better how tumour derived DNA is released into circulating blood and to develop strategies that could optimise this process for the purposes of developing new cancer diagnostic tests.
What species and approximate numbers of animals do you expect to use over what period of time?	The project will use exclusively mice and will run over five years. During that period we expect to use up to 85,000 animals. Of these, no more than 25,000 will be used in experiments involving experimental interventions, such as drug treatments, with the others required mostly to support the breeding programme of the complex genetic traits underling the cancer prone animals.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Tumour prone animals will develop cancers that will eventually cause their death if there is no further intervention. To minimise suffering for these animals, stringent checks will be in place to ensure that mice are culled as soon as signs of suffering or distress as a result of their cancer or its treatment are detectable. Only tumour bearing animals that are healthy will be treated with drugs or subjected to non-invasive imaging to address the experimental aims of this programme. Very rare instances may occur where rapid progression of the cancer could result in death from the disease, or its treatment, before any signs of

	suffering were detectable, despite stringent monitoring. It is expected that the rate at which this would occur would be no more than 5% of the animals bearing tumours in internal organs in experimental drug treatment studies, and considerably lower for other animals. Our aim is that animals will be culled at the end of the defined experimental period and before severity limits are reached.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have extensively used cultured cancer cells in the run up to this project. We have also used bioinformatic analysis of publicly available data from cancer genome sequencing studies. However, various aspects of the cancer disease process can only be addressed in living animals. The development and function of the immune system, which is a focus of the first part of our work, involves many different cell types that cannot be mimicked in vitro. Similarly, the release of DNA from tumour cells into the blood involves a plethora of interactions between cancer cells and surrounding tissues, which is unlikely to be achievable in vitro.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have used in vitro cancer cell culture systems to define a limited set of hypotheses that merit testing in animal models. Mouse breeding experiments have been planned in detail in consultation with experts in statistic and animal breeding. We will ensure that the minimum numbers of animals are used to obtain statistically meaningful results. Mouse colonies will be actively managed to ensure that the basic principles of mouse breeding will be adhered to and only the minimum number of animal required for the experiment are generated. Transgenic lines will be frozen down wherever possible rather than keeping breeding stocks. In addition, the use of in vivo imaging methodologies greatly reduces the number of animals needed compared with end point assays as each mouse can be followed over time and inter-

	mouse variability is internally controlled for.
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 one of the model organisms that most closely resemble humans. Mice can be genetically altered and have been extensively used for the topics of our investigation. The mouse cancer models we will use are internationally regarded as the best available for the accurate modelling of the human disease, for example by the US National Cancer Institute (http://emice.nci.nih.gov/aam/mouse). The mouse model systems chosen allow studies of relevance to the 20% of human cancers in which activation of the RAS oncogene has been implicated. Only by allowing tumours to develop in these mice can we address the importance of the molecular interactions that we are seeking to investigate in this setting. The severity of the procedure will be limited by ensuring that animals are killed as soon as overt signs of the disease can be seen, and that mice are rigorously monitored for signs of suffering or distress at all times. These studies address the response of tumours to experimental therapies, but have been designed to focus on mice with early stage disease, in which setting the impact of the tumour on the overall health of the animal should be small. Live imaging of the animals, such as by CT scanning, will be a major feature of these experiments which will allow improved data collection from smaller number of animals; this will be carried out under anaesthesia to minimise distress to animals. Drug treatment will involve only the use of agents that have already be tested on mice, so will not involve chemicals where unexpected toxicities are likely to occur.

<table>
<tr><td>PROJECT 26</td><td>Investigating cellular protein production</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>cancer, protein production, gene expression.</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Humans are made from trillions of cells. Each cell contains many proteins which have functions including, providing energy, carrying oxygen providing structure, making other proteins, and creating movement. My lab is interested in how proteins are made in the cell.

Proteins are made in the cell by a complex series of chemical reactions. DNA carries the template for making a substance called RNA and RNA is the template for making protein. RNA is heavily processed after being made to make it competent to be the template for protein. We know that this processing is important, since if we prevent it cells die.

Our objective is to answer the following questions:

1. What is the mechanism by which RNA is</td></tr>
</table>

	modified? 2. How does the cell influence this RNA modification process to change the rate at which different proteins are made? 3. Can we kill cancer cells by interfering with how proteins are made?
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 mechanism by which proteins are made is fundamental to human biology. Human diseases such as cancer and neurodegeneration are often caused when the mechanism of protein production becomes deregulated in the cell. Therefore determining the mechanism by which RNA is processed is likely to directly or indirectly impact on medical research.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to breed about 12500 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 level of severity? What will happen to the animals at the end?	We shall need to harvest cells and tissues from some of the mice, *after* they have been killed humanely. In order to generate animals with the required genetic alterations, others may have to bred and then not used. These too will be killed by humane methods. The administration of tissue-labelling compounds to some animals in the few days before they are killed is not expected to cause them any harm. The great majority of the animals that are bred are expected to display essentially normal welfare throughout their lives. However, some genetic alterations *might* cause harm. We shall there observe all animals very closely and any in which there appears to be a welfare problem will be killed humanely before it becomes more than moderate. Most of the experiments proposed use cells from a euthanised animal. Animals will be euthanised using a schedule one method. We will perform a limited number of experiments to delete genes of

	interest. We expect such mice to die before birth.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We predominantly use non-animal alternatives in our research programme. However cells cultured outside animals acquire genetic changes over time which mimic the changes that occur in cancer cells. For this reason we need to use cells directly taken from mice to understand how normal tissues work.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will manage our breeding colonies very carefully. In particular, we will adopt the most efficient routes to the generation of the genetically altered animals that we need, provided these are consistent with good animal welfare.
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, since our research shows that the capping enzymes have unique configurations and functions in mammals. It is also possible to generate animals with specific alterations in the genes for these enzymes. Tissues and cells harvested from these mice can then be studied in detail to understand more fully the role of the messenger RNA cap. As above, we will balance breeding efficiency very carefully against animal welfare in order to minimise welfare costs. We expect the great majority of mice to experience essentially normal welfare throughout their lives.

<table>
<tr><td>PROJECT 27</td><td>The phosphoinositide-network in health and disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Inflammation, cancer</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>No</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>To find new ways to treat diseases such as chronic inflammation and cancer and to define factors that determine health span through better understanding of cellular molecular mechanisms.

All of life can be viewed as based on molecules and chemistry. To be able to understand and treat human health and disease we need a molecular understanding of biological processes because it is only at this level we can meaningfully and rationally attempt to use “designer molecules” as therapeutics. Our work aims to provide a molecular “chemical” understanding of the biology involved in inflammation and tumour progression and to identify ways to treat disease with minimum unwanted “side effects”.</td></tr>
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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)?	Better understanding of the molecular mechanisms underpinning health and disease. The validation of new approaches to treat socio-economically important diseases such as inflammation and cancer. We work closely with drug companies to improve therapeutic strategies.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, approximately 13000 per year.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The large majority (over 95%) of animals will experience mild suffering at worst, the main adverse effects could be mild transient inflammatory responses. Approximately, 4.5%, will experience moderate suffering at worst, the main adverse effect could be a more sustained inflammatory response in some mice the main adverse effect could be lethargy and weight loss associated with early tumour growth. Under 0.5% will transiently experience severe suffering at worst. The main adverse effect will be a strong inflammatory reaction. All animals will be killed by a quick humane method at the end of the experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We use many approaches that allow us to avoid the use of animals in research, including use of cell lines. However, to understand healthy processes and what goes wrong in disease and to devise strategies to treat disease, some use of animals, that are similar to humans in terms of their normal cellular processes and their responses to specific diseases, is necessary.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use many tactics to reduce the number of animals we need to use. By employing good statistical methods, by using modern technologies that minimize error, using techniques that allow us to study mice non-invasively (and therefore to be able to make many measurements with the same animal) where 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.	Mice are the best species to use for the objectives of this licence. They have many similarities to humans in terms of their basic cellular processes and responses to diseases and as they are very widely used in academic and pharmaceutical research, results obtained in mice are easily compared to those from other research groups. Many highly evolved and technically efficient methodologies have been optimised with mice leading to more efficient progress per animal used. We only chose to work with models that are widely accepted to be reliable and have been optimized to minimize harm and the number of animals used. We use non-invasive techniques as much as possible and attempt to remain in touch with new advances that offer further animal-welfare advantages. Our animal work is done within the framework of a limiting clinical signs approach, operated by animal technicians and vets; that is, any mice seen to be experiencing unexpected suffering are killed by a humane method.

<table>
<tr><td>PROJECT 28</td><td>Understanding the molecular basis for invasion and metastasis of melanoma and pancreatic cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cancer metastasis, pancreatic cancer, melanoma</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>We seek to understand the molecular basis for cancer metastasis (spread in the body). Cells have a skeleton just like bodies do and the cell’s (cyto)skeleton helps it to move. We study the basic molecules that make up the cytoskeleton and we apply this knowledge to understand how cancer cells move from a primary tumour to another site in the body.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>We hope to uncover new targets for development of medicinal therapies against the spread of cancer. Our project concentrates mainly on melanoma and pancreatic cancer, but our work applies to many cancer types. We also hope to understand more about the basic mechanisms by which cells in animals move and function.</td></tr>
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What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use up to 6,000 mice per year over 5 years for this project. Around 80% of these will not undergo any scientific procedures, but will be used solely for breeding and maintenance of colonies.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Animals will be bred to show predisposition to melanoma or pancreatic cancer or will receive a transplant of tumour tissue or cells from mouse or human cancer. Approximately 80% of the mice will not show any adverse effects related to the breeding and not undergo any procedures except for ear notching for identification and genetic testing. These will be kept in normal housing and humanely killed when they are no longer needed for breeding. We will often be able to use tissue samples from these mice after they are killed as normal controls. Some proportion of the animals (approximately 20%) will be predisposed to cancer and will be monitored carefully for clinical symptoms. Symptoms include weight loss, swelling of the abdomen and development of visible or palpable tumours. Mice with tumours will be monitored carefully by trained staff and if their symptoms reach a moderate level, they will be humanely killed and the tissues will be analysed. Tumour cells will be grown in the laboratory. In some cases, we will treat animals with experimental chemical compounds and measure the effects on tumour growth or spread. This may involve adding substances to the food or drink or injection of substances. In a very small proportion of cases (likely up to 1% per year), we will treat the mouse with a terminal anaesthetic and observe the tumour tissue under the microscope to observe cancer cells moving in live tumours. All animals receiving treatments will be monitored closely and any animals exhibiting moderate symptoms will be humanely killed. At the end of the study, all animals will be euthanized.
Application of the 3Rs	
1. Replacement State why you need to use	Cancer metastasis can only be accurately modelled in an animal, as cancer cells encounter various

animals and why you cannot use non-animal alternatives	organs and tissues. Mouse represents the best model for human cancer available to us, due to the ability to manipulate the DNA and test the effects of loss or alteration of specific genes on cancer progression.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We design our experiments to use the minimum numbers of animals that will give statistically significant and useful results. We also seek to share animals between experimental groups when possible. We perform pilot experiments using only a few animals for new studies, before scaling up to the appropriate numbers for a full study. Most of our work is done with cell cultures or tissues taken from animals that have been humanely killed, to minimise the amount of work done with live 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.	Mouse genetic models of cancer are widely accepted to be the most closely representative of human cancers. The tumour forms in the correct tissue and spreads via the normal routes and the tumours often progress through the same stages of pre-cancer as in humans. We use state-of-the art genetic models to ensure that the cancer develops in the correct organ/tissues and there are as few side effects as possible due to breeding or treatments. This is done with inducible DNA recombination enzymes that are specific to the target tissues of interest and is achieved by breeding these into the genome. Animals will receive anaesthetic and/or analgesic treatments where appropriate. All animals will be monitored regularly for signs of normal behaviour and will be humanely killed if they exhibit moderate adverse signs.

<table>
<tr><td>PROJECT 29</td><td colspan="3">PTEN and the PI 3-kinase signalling pathway</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Cancer, signalling, pten, tumour suppressor</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Cancer is driven by the accumulation of genetic changes that transform normal cells into tumour cells. There is great diversity amongst the genetic changes that accumulate in tumours, with differences being observed between different cancer types and even between tumours that arise in the same tissue and might appear similar. However, there are a few functional pathways that appear to be important in the formation of many types of tumour, and it is common that cancers have a mutation in at least one component of each. Mutation of a gene called PTEN is one of the most common changes in cancer. The function of PTEN is impaired through mutation in around a quarter of all cancers. There are many different downstream pathways activated by PTEN loss, some of which can be blocked by drugs. However, it is currently</td></tr>
</table>

	unclear which of these downstream pathways activated are key to driving tumour formation in tumours lacking PTEN.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We aim to identify specific biochemical signalling mechanisms that drive tumour formation. This information should assist in developing, and selecting patients for, drugs targeting components of the PI3K signalling network.
What species and approximate numbers of animals do you expect to use over what period of time?	A maximum of 6000 mice over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	In almost all cases of spontaneous tumours and subcutaneous xenografts, the adverse effects of small tumour formation are expected to be modest. Spontaneous tumour formation in Pten transgenic mice has been well studied with most mice developing lymphomas that are externally evident due to swollen lymph nodes. In a few cases, internal tumours may only be detected through effects on the broader health of the animal, e.g. weight loss, and these would be considered moderate severity. In all cases, animals will be humanely sacrificed if any adverse welfare is detected. In experiments to study the formation of tumours, animals will be humanely sacrificed if a tumour is identified from external examination, or if the existence of a tumour is suspected due to any unexpected signs of ill health, such as weight loss or reduced activity. In some experiments used to test whether drugs can stop tumour growth, treatment is only started once small tumours have been identified underneath the skin. In these cases, some tumours may be allowed to grow until they reach a certain size and only if they do not otherwise affect the health of the animal.
Application of the 3Rs	
1. Replacement	Experimental methods not involving animals are used whenever possible, as shown by our

State why you need to use animals and why you cannot use non-animal alternatives	publications. However, the complexity of real tumours means that current alternatives do not come close to replicating the many factors that influence tumour formation in an animal. The results obtained using cultured cells are too often different from those obtained using animals and in studies of human patients. Specifically, the mice that we study get almost the same range of tumours as human patients with the same genetic defects, showing us the closeness of the two circumstances.
2. Reduction Explain how you will assure the use of minimum numbers of animals	When planning projects, we will use statistical power calculations to determine the minimum numbers of animals required to generate significant data and during active projects, experiments will be stopped and breeding ceased once enough animals are obtained. In all cases we will attempt to use the minimum number of mice and obtain maximum value from each animal, for example by performing multiple forms of analysis on the same tissues.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice have been heavily studied as models for human cancer and many transgenic lines are available that allow us to extract the most information from each experiment. All mice will be sacrificed humanely at the earliest possible stage. Most tumours are externally visible and will not need to grow large enough to cause any problems to the animals welfare.

<table>
<tr><td>PROJECT 30</td><td>Cancer biology including host immunity in zebrafish</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cancer, immunity, zebrafish</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td></tr>
<tr><td>Regulatory use and routine production</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>To learn more about cancer induction, maintenance and progression, including evasion of host immunity, focusing on melanoma and pancreatic neuroendocrine cancer</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>1) The research will identify and validate novel drug targets for the focus cancers: cutaneous and uveal melanoma and pancreatic neuroendocrine cancer. Drug targets are the entry points for rational drug discovery and target identification is the rate limiting step in the drug discovery process.

2) The research will expand our understanding of the function of the immune system in fish, and indicate whether it is suitable for research into human disease. The research could potentially benefit the fish aquaculture industry which is trying to improve disease management in fish stocks through developing vaccines, which requires</td></tr>
</table>

	knowledge of immune system function. 3) Assuming we are successful with our efforts to advance basic understanding of the function of cell-mediated immunity in zebrafish, subsequent research could uncover what limits host immune responses to cancer. 4) The research may validate novel radioprobes that can be used in cancer diagnosis together with positron emission tomography (PET)
What species and approximate numbers of animals do you expect to use over what period of time?	45,000 zebrafish over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Zebrafish will be genetically modified and this may lead to genetic disease or cancer which could incur severe suffering (although zebrafish have much lower neurological complexity than mammals) in a limited number of animals. This will be mitigated by frequent inspection and early intervention. Zebrafish will also be vaccinated which is assumed to elicit only mild irritation
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The involvement of multiple cell types in the process of cancer formation and progression is impossible to reconstitute in vitro or in invertebrates.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Exploratory experiments, where possible are first performed in vitro or in embryos (which are considered non-sentient). Efficient experimental design and statistical techniques such as power analysis will keep the number of protected animals used to a minimum.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	Zebrafish is the vertebrate of lowest neurological complexity that can be genetically modified to produce the required alterations. Animals will be sacrificed as soon as tumour formation is sufficient to yield the desired data streams, which will anyway

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	be before tumours reach a size that can interfere with feeding, locomotion, respiration or cardiovascular function, or induce significant behavioural or other physiological abnormality. Likewise any genetic manipulation indicated to interfere with feeding, locomotion, respiration or cardiovascular function, or inducing significant behavioural or other physiological abnormality will result in immediate termination of the organism concerned and other animals sharing the genotype.

<table>
<tr><td>PROJECT 31</td><td>The Tumour Microenvironment in Cancer Progression</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cell death, Inflammation, lymphoma</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>(1) To understand how tumour cells (which carry the genetic mutations of cancer), especially dying tumour cells, interact with the normal host cells that are always found in cancer tissue; (2) to determine how these interactions lead to cancer progression; (3) to identify potential diagnostic or therapeutic targets.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The research will provide much-needed information about the microenvironment of aggressive malignant tumours that will help improve prospects for early diagnosis, cause, outcome and treatment of cancer. This will be of importance both to human and to animal healthcare.</td></tr>
<tr><td>What species and approximate numbers of</td><td>Approximately 3000 mice, 4000 zebrafish and 20</td></tr>
</table>

animals do you expect to use over what period of time?	rats over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Inbred (normal) and genetically altered animals will be bred to study the mechanisms of tumour formation through transplantation of tumour cells from the laboratory to the animal or through spontaneous tumour formation. Because tumours will not be allowed to grow to a large size, adverse effects are expected to be mild or moderate. Animals will be sacrificed humanely at the end. Harms to the animal will be mainly through injections which will only cause transient and mild discomfort.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The tissue environment of tumours is complex and it is not possible to study it without using animals. It cannot be recapitulated in the laboratory by cell or organ culture, although certain aspects of the tumour environment can be studied using simplified cell culture models, such as co-culture of tumour cells with leucocytes *in vitro*.
2. Reduction Explain how you will assure the use of minimum numbers of animals	This research group is highly experienced in the experimental models that will be used. Sample sizes will be minimized through rigorous experimental design and statistical principles. Reduction in animal numbers will also be achieved where possible via 'pre-screening' protocols – such as exposure of tumour cells to a specific reagent or cell – so that candidate mechanisms can be identified in the laboratory through simplified cell culture approaches.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs	Mice are the mammals of choice for studies of cancer biology because of the number of tools available (such as important genetic variants of laboratory strains) and because of the established tumour models. These models are highly relevant to our work because they show the cellularity and tissue architecture of their human counterparts and so are very relevant for improving healthcare,

(harms) to the animals.	ultimately. Rats will only be used as appropriate to produce antibodies against mouse molecules where raising antibodies in mice is not possible (antibodies will be used to test the targeting of specific molecules which may constitute future therapeutic targets). Because of their small size and optical transparency, zebrafish provide excellent genetic models which are particularly suitable for real-time cell imaging studies, especially of the early stages of cancer development. However, mammals such as mice also need to be used since fish are too far removed from humans to provide a comprehensive animal model for mechanisms relevant to human cancer. Furthermore, many tools (such as reagents for phenotyping of cells) are immediately and readily available for use in mouse models and these are not available for zebrafish systems. All protocols are well-established and known to produce mild or moderate adverse effects and personnel involved with the animal work have substantial expertise in carrying out the specified protocols and in observing (and responding appropriately to) adverse effects. Strict humane endpoints will be applied throughout.

<table>
<tr><td>PROJECT 32</td><td>Mechanisms of normal and leukaemic haematopiesis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Haematopoietic stem cells; leukaemia; haematopoiesis.</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Regulatory use and routine production</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>~~Yes~~</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The general goal of this research program is to understand mechanisms which control the normal blood and leukemic stem cell functions. The specific objectives of this proposal are:

1. To determine functions of candidate regulators of normal blood stem and progenitor cells;
2. To explore how genes found to be altered or mutated in human leukaemia contribute to the development of disease;
3. To identify and characterise different cell types that initiate human leukaemia and cells that resistant to therapeutic treatment.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or</td><td>These results will contribute both to an improved manipulation of blood cells for therapeutic use and to an understanding of disease mechanism. Our studies may also identify novel therapeutic targets</td></tr>
</table>

animals could benefit from the project)?	for improved treatments of blood disease and potentially other forms of cancer. These studies should also provide insight into the regulation of hematopoietic stem cells, a type of rare pluripotent cells which reside in bone marrow and produce mature blood cells throughout an animal's life time. Eventually, these results might be used to reduce the morbidity and mortality following bone marrow transplantation.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice only and we estimate that a maximum 34,500 mice will be used over the 5 years of PPL (2014-2019).
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?	Our overall plan of work can be divided into the following two parts: The first part of the projects includes the breeding and use of transgenic mice. Tissues or cells from transgenic animals or treated animals will be harvested and analysed *in vitro* using cell and molecular approaches to understand the functions of candidate regulators in normal blood stem and progenitor cells. The second part of our project includes experiments where it will be necessary to assess the behaviour of the cells also *in vivo*, as *in vivo* study still represents the gold standard assay for stem cell function. In this event, cells will be transplanted into recipient mice, a similar procedure as bone marrow transplantation in patients with leukaemia. We will monitor the engrafted cells to understand the development of normal or leukaemia cells. Once the engraftment is established, we may then carry out a therapeutic treatment for these mice to study the characteristic and mechanism of those resistant cells. The transgenic mice we used in this project are not expected to exhibit any significant harmful phenotype although many are likely to have some impairment of their immune system and may succumb to infections not affecting normal mice.

	They will be kept in pathogen free status within barrier systems to protect them from infections. Animals carrying transgenes, mutations or with transplantation may develop leukaemia or high grade lymphomas. In this case leukaemia can be recognized by elevated white blood cell counts, or similar symptoms observed in patients. Close observation of animals will be used to identify the onset of symptoms associated with a developing tumour burden. However, it is not possible to fully predict the nature or severity of any potential defect and for all types of mice there will be careful monitoring for possible side effects. Animals exhibiting any unexpected harmful phenotypes will be humanely killed, or in the case of individual animals of particular scientific interest, advice will be sought from the Named Animal Care & Welfare Officer (NACWO) and facility vet.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have explored the possibility of experimental methods that do not require the use of animals at all or do not require live animal experimentation. As a consequence, much of our research is carried out in culture with normal cells obtained from healthy human volunteers or culled mice. In order to replace animals with *in vitro* models we will use human stem cells isolated from umbilical cord blood. We will also use cell lines, which are derived from mouse bone marrow and can be grown in culture to investigate some of the key features of stem cells. To investigate developmental processes we will employ embryonic stem cell lines instead of using mouse embryos.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The majority of mice used in this project will be generated by breeding of transgenic animals. In order to establish functions of genes of interest in stem cells we typically compare cell functions in wild-type and mutant mice. As most of assays in stem cell biology require purification of a rare

	population of bone marrow-residing stem cells, in many occasions, we need to pool bone marrows from several mice together in order to obtain sufficient number of stem cells for further studies. Therefore, the numbers of mice used in breeding protocol reflect technical challenges of working with small populations of cells. The smallest number of mice required for each experiment will always be applied. These numbers are based on past experience and on theoretical calculations. Assays requiring cells from animals will be carefully optimized in order to minimize the number of animal cells required. Another important means of achieving reduction will be to apply most efficient breeding strategies. We will replace breeders before their reproductive performance declines. Non-productive breeders will also be replaced. We will regulate the breeding depending on needs; if we predict that offspring from a particular litter will not be required for several months, we will adjust the number of breeders accordingly.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are chosen because they are the lowest form of mammal available for study and are the most frequently used model system to study biology of stem cells and cancer. Other advantages of the mouse model include the availability of antibodies for the identification and purification of different classes of stem cells and mature blood cells and the availability of *in vitro* and *in vivo* functional assays. To ensure technical competence, the staff performing the experiments will be fully trained and directly supervised by myself or senior postdoctoral fellows who have extensive experience in experiments on animals. To minimise infections of immunocompromised mice, where appropriate, the animals will be housed in individually ventilated cages or in conventional cages with filter tops. Cages, food, water and bedding of immunodeficient animals will be sterilised. If mice are in pain,

	analgesic will be given. Transgenic animals exhibiting any unexpected harmful phenotype will be humanely culled, or in the case of individual animals of particular scientific interest, advice will promptly be sought from the local Home Office Inspector and veterinarians. All the work involving mice with leukaemia will be undertaken in accordance with the principles set out in the National Cancer Research Institute (NCRI) Guidelines.

<table>
<tr><td>PROJECT 33</td><td colspan="3">Understanding the repair of damaged DNA</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">DNA damage; repair; cancer; chemotherapy</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">The DNA in every cell of our body is an instruction manual for the normal functioning of cells. A major problem is that DNA is constantly under attack from agents that arise inside and outside the cell that cause DNA damage that can cause changes or “mutations” in the DNA sequence. Ordinarily, mutations occur at very low levels because healthy cells have the remarkable ability to detect and eliminate DNA damage. However, DNA damage sometimes leads to a gradual accumulation of mutations in cells, leading to “re-writing” of the instructions encoded in DNA. These changes underlie a wide range of human disease including cancer. From a clinical perspective many commonly used chemotherapeutic agents (anticancer drugs) act by inducing DNA damage and/or DNA replication stress and we are interested in finding ways of making these therapies more effective and in preventing resistance. Furthermore we are</td></tr>
</table>

	involved in identifying new anti-cancer drug targets in the DNA repair arena.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	There are many potential benefits that could derive from this project. These include: advancing our understanding of DNA repair and how cells prevent mutations that cause disease; advancing our understanding of the role of DNA repair in diseases such as cancer; pinpointing promising new anti-cancer targets and anti-cancer drugs. We already developed Fan1 small molecule inhibitors and the current proposal will allow is to validate the potential of these drugs for treatment of cancers.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice; approximately 8,000
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Almost of the planned experiments will be carried out on cells derived from embryos so that we do not need to do experiments on animals. In that sense most of what we propose to do simply involves the breeding of genetically altered animals. The GA animals we have already made show no signs of adverse welfare. In the case of genetic alterations we propose to make, adverse effects are not anticipated but a very careful check will be kept.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We use human cell knockouts to test gene function but these analysis provide limited and often misleading and inaccurate information because cultured immortal cell lines are genetically unstable.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All experiments will be subjected to rigorous statistical methods to keep animal numbers to the bare minimum. Most of our experiments will be carried out on cells instead of animals.
3. Refinement Explain the choice of species and why the animal model(s)	Mice are mammals, like humans, and we hope to learn a lot about the functions of the genes we work with in the mouse system. None of the mouse strains we currently work with, and intend to

you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	continue working with, have any welfare issues. We do not anticipate any harmful effects of the alterations we would like to make in the future. In any case, most of the work will be carried out on cells from embryos and consequently we will not need to generate large numbers of animals. We will keep a close check on mice with genetic alterations in genes of unknown functions, and animals showing the slightest signs of adverse welfare will be culled until an appropriate course of action is decided in consultation with the vet and NACWO.

<table>
<tr><td>PROJECT 34</td><td colspan="3">Mechanisms of cancer development</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Cancer, heterogeneity, stem cells, epigenetic</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 yrs</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">The overall aim of the study is to identify and characterize cellular mechanisms important for cancer development, with the ultimate goal of using this knowledge to develop novel strategies to treat the disease. To this end, we mainly focus on studying cancer stem cells, a particularly important subset of cancer cells which play critical roles in the formation and maintenance of tumours.

Cancer is a leading cause of death worldwide and limited progress has been made in improving patient survival over the last 40 years. A major challenge in fighting the disease, is dissecting cancer heterogeneity. Every patient is different, but even within the same patient, a tumour comprises various subpopulations of cells with distinct biological properties. Even neighbouring cells within a tumour may have different shapes and express different genes. Most importantly, not all cancer cells can divide in same way and in most cancers</td></tr>
</table>

	only a subset of cells is truly immortal. These cells have been named cancer stem cells (CSCs) and are those which give rise to the rest of tumour cells and sustain the long-term growth of tumours. Often, these cells are resistant to conventional chemotherapy and are responsible for cancer relapse, which in many cases leads to patient death. Understanding how these cells function and what makes them different from the chemotherapy-sensitive cancer cells is critical to design more effective strategies to treat the disease. Very little is known about CSC biology and we aim at shading light on the mechanisms that regulate their function within a tumour.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The primary potential benefit of the proposed study is to increase our knowledge of CSC and tumour biology. The information is likely to be directly relevant for pre-clinical studies focused on tumour biology and assist the design of future anti-cancer agents. In addition, our study may assist oncologists in cancer prognosis. Identification of CSC markers will allow a more accurate diagnosis of the stage of the disease, evaluation of response to therapy and chances of relapse, and overall a better clinical management of patients.
What species and approximate numbers of animals do you expect to use over what period of time?	For our study we will use mice, the best model system to study cancer biology. We expect to use about 40,000 mice over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	In this project we will mainly create tumours in mice where specific conditions are altered, trying to understand how deregulation of gene expression leads to CSC formation and how CSCs control the long-term growth of tumours and lead to cancer relapse. To achieve these aims, a variety of well-established experimental techniques will be employed, including breeding of mice which will develop tumours spontaneously, induction of tumour growth by injecting tumour cells or exposing animals to chemical or biological agents, and induction of skin tumours using transplantation

	chamber. In most cases (~70%), tumours will be superficial and will not affect organ function, impacting only minimally on the animal overall condition. On rare occasions only, minor surgical procedures, such as skin biopsies or resection of small tumours will be carried out. Non-invasive in vivo imaging will also be performed under general anaesthesia. At the end of the experiment, animals will be humanely killed and tumours harvested. Overall, we expect mild (protocol 1) or moderate (protocol 2 and 3) adverse effects associated with the protocols used in this project. To be able to achieve our scientific objective, about 50 % of the animals used in protocol 2 will need to grow tumours to a size larger than that recommended by the NCRI guidelines. This is necessary because one of the main purposes of protocol 2 is to induce tumour formation with the goal of producing, in a controlled fashion, high numbers of CSCs for *ex vivo* analysis. To allow proper differentiation of cancer cells and ensure that enough cells will be available for analysis after tumour removal, it is necessary that tumours grow to 2 cm in diameter over a 2-months period. However, we have previously seen that such large tumours cause no more than moderate pain or distress to animals, since they are typically very superficial and do not affect organ function. Due to superficial nature of the induced tumours, up to 30-40% of the animals may develop small ulcerations. We have extensive previous experience with similar experiments and have seen that small ulcerations most of the times do not cause severe pain to animals. The use of suitable analgesia will help minimizing animal suffering. To be able to achieve our scientific goal reducing the number of animals needed to obtain statistically significant results, animals developing ulcerated tumours will not be euthanized, provided that this does not cause severe pain to the animals. We will monitor animals twice a day using a scoring system to objectively assess animal condition. For most of the animals

	we expect only moderate adverse effects, but if signs of ill health are observed, animals will be culled immediately.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The *in vivo* experiments will tightly interconnect with *in vitro* and *ex vivo* experiments. To partially replace the use of animals, we have developed a cellular experimental system which models *in vitro* the early phases of cancer development. Most of the work investigating CSC formation will be done *in vitro*. Studies focused on understanding mechanisms regulating CSC function within established tumours and their contribution to cancer relapse will necessarily require more *in vivo* experiments, since we need to analyse tumours in the context of the tumour microenvironment, and the complexity of the process cannot be modelled *in vitro*. However, most of the experimental measurements and the analysis of tumours will be performed ex vivo post mortem.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our use of in-vitro approaches limit the numbers of animals required for the in-vivo investigation stage. In addition, when dissociated tumour cells are not in use, they will be stored in a frozen state. This minimises the numbers of animals required for maintaining live tumour cells. When animals are needed, we employ several strategies to try to limit the number of mice in the study. Firstly, we always aim to maximise the amount of data we get from each mouse, for example by injecting tumorigenic cells in both flanks to induce two tumours/mouse in xenograft assays. Also, we will limit the use of genetic models (that often require many generations breeding) using transplants of cells and treating the mice with chemical agents to generate tumours. We also use the minimal amount of mice needed for statistical significance when testing the experimental hypothesis. Furthermore, we will use in vivo imaging, which allow to use the same animal for repeated measurements and reduces the overall number of animals needed.e Finally, by careful

	monitoring of our mouse colonies we try to breed as few mice as possible. We will also collaborate with other groups, sharing data and animal tissues, in order to minimize the overall number of animal used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We will use mice, the best model for studies focused on cancer biology. The early stages of the project will mainly use immunocompromised mice which allow tumour formation by injection of human cells. Animals will be housed in highly clean facilities to minimize the chance of infections. Tumour studies using genetically modified animals will be mainly performed using transplants of cells and treating the mice with chemical agents using well-established and refined protocols. When performing procedures in which we are not fully competent, we will seek the help of other groups in the institute that have optimized those procedures. 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. The use of specific treatments (when possible) or methods of humane killing will be used depending on need. Surgical procedures, when needed, will be done with suitable anaesthesia and animals monitored post-surgery to ensure that they recover well. We will also use suitable analgesia according to the procedure.

<table>
<tr><td>PROJECT 35</td><td>Development and refinement of Small Animal Imaging</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Imaging, Development, Biomarkers</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>This project aims to improve existing small animal (rats and mice) imaging and develop new imaging techniques to be used by researchers and other Project Licence holders. A positron emission tomography (PET) scan is an imaging test that helps reveal how your tissues and organs are functioning. A PET scan uses a radioactive drug or molecule (tracer) to show this activity. The tracer is injected and the tracer collects in areas of your body that have higher levels of chemical activity, which often correspond to areas of disease. On a PET scan, these areas show up as bright spots. A PET scan is useful in revealing or evaluating several conditions, including some cancers, heart disease and brain disorders.
Magnetic Resonance Imaging or MRI provides information on structural and bodily changes within a living organism. It can be used to look at the progression of disease e.g. brain disorders or brain cancer. The agents used to visualise disease or highlight bodily structures need to be developed</td></tr>
</table>

	and improved to ensure they are being used correctly. New imaging techniques will also be validated for routine use in the imaging laboratories. Imaging biomarkers (chemical or bodily changes that can be detected by imaging) are important for diagnosing disease, monitoring its progression, tracking response to therapy and building knowledge of physiology. We need to understand the changes in imaging biomarkers during an treatment to ensure that we interpret data correctly Such methods minimise the number of animals required in preclinical research since each animal can be scanned again and again over time and each animal can be used as its own control (e.g. baseline scans). This avoids the need for killing groups of animals at fixed time intervals. These imaging techniques fully utilise the 3R's philosophy. In a majority of studies and where possible preliminary work will have been carried out using in vitro cell culture methods (PET) or in cadavers or phantoms (MR)
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 increase our understanding of imaging readouts and will allow continued improvement of animal imaging protocols and will help overcome the challenges common to all imaging techniques including (a) the design of tracers/probes that are specific to the biological process e.g. disease of interest, (b) improvement of imaging systems to provide the highest sensitivity image quality, (c) minimising the disturbance to the biological processes under observation so that the experimental outcomes relate to the biology and not the probing d) quantitation of the imaging signal and looking at its relationship with an underlying biological parameter. Non-invasive imaging offers a substantial improvement enabling repeat scanning of the same animal and subsequent reduction in numbers. Investigation of Imaging Biomarkers in response to treatment may help us to understand the changes in biology and could ultimately lead to the identification of clinically relevant biomarkers could potentially aid patient selection and disease monitoring which can have considerable benefit for patients, in terms of preventing the need for unnecessary treatments or allowing changes in treatment as soon as one is identified as "failing"

	and in terms of financial costs.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats 600, Mice 900 over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Many experiments will be carried out under non-recovery anaesthesia and therefore the most likely adverse effect expected is anaesthetic overdose which is controlled by monitoring the animal during prolonged periods of anaesthesia. Tumour growth is occasionally associated with a reddening of the skin in superficial tumours. Maximum size is restricted to below 1.25cm^3 that equates to less than 5% body volume. For new xenograft models or more complex disease models, Initial pilot studies will have been carried out on other PPL's to determine model progression/prognosis and to identify early indicators of a decline in well-being that could be used as an end-point indicator in subsequent studies on this PPL (for example general loss of condition or weight that occurs before a severe adverse event). Imaging is used at early time points in disease progression prior to any detrimental effects as a consequence of disease burden. Imaging is also incorporated, if possible, where tumours are not palpable (brain tumours) to monitor size. Maximum size is restricted to below 1.25cm^3 that equates to less than 5% body volume. Other adverse events that could occur are: Treatments used can cause weight-loss and in superficial tumours, skin reddening or scabbing at the tumour site. Ulceration which is characterized by a weeping open wound is very rare and would indicate mouse cull. In all cases the animals are culled at the end of experiments. Models of Brain tumours and neuroinflammation may be transferred from other PPL's (Continued Use). Animals will be closely monitored for signs of disease progression

	and adverse effects. At the end of a series of regulated procedures the animals will be humanely killed by either a schedule 1 method or using a recognised method to collect blood or preserve tissue for analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Many of our objectives can be achieved in phantoms, fruits and vegetables, or cadavers, or in humans. Some of our objectives can be achieved in human volunteers or patients, but in many cases, in accordance with the declaration of Helsinki, it is unethical to perform human studies before suitable animal studies have been performed. Physiologic function (heart beating, lung and diaphragm movement) is required to develop/improve animal imaging protocols as physiologic function itself can have a undesired effect on the imaging readout. Other non-animal techniques such as Liquid chromatography/mass spectrometry (LC/MS), combined with commercially available hepatocytes, has become an indispensable tool in evaluating the presence of these metabolites in target tissues, however some tracers do not produce good metabolites *in vitro* and *in vivo* studies must be used.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Imaging can use each animal as own control, allowing paired comparisons, thus increasing statistical power of experiments compared with terminal designs. Imaging studies are inherently sequential (only one animal can be scanned at any one time), so lend themselves to adaptive designs, which use fewer animals to achieve the same statistical power as conventional designs. Tumour Studies will utilise tumour models that have robust, consistent growth characteristics. The implant of cohorts for the imaging study will be

	staggered over time, with generally 4 mice imaged per day depending on tracer. This is repeated until the experimental group sizes are achieved. Archived imaging data is used to refine sample size calculations enabling a reduction in group sizes.
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.	Rats and mice are essential mammalian species as they cover the great majority of well characterised disease models, they are well understood which means that results are likely to be interpretable. They will be used as they represent the species with the lowest neurophysiologic sensitivity and the protocol will be one that causes the least pain, suffering, distress or lasting harm and that the results could not be achieved by any other reasonably practical method not using protected animals. Imaging allows the measurement of small changes in animals that may be clinically normal. This provides a refinement in comparison with clinical endpoints in clinically abnormal animals. Even where animals are clinically abnormal, it is often possible to use milder disease than with other assessments Where possible, mice will be used in preference to rats. Where possible, normal animals will be used in preference to tumour-bearing animals. Where possible, terminally-anaesthetised animals will be used.

PROJECT 36	**Novel therapies for malignant germ cell tumours**	
Key Words (max. 5 words)	MicroRNA, germ cell tumour,	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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)	Malignant germ cell tumours (GCTs) are one of the leading causes of cancer-related death in young men worldwide. There are —2,500 cases of the testicular form of the disease in the UK alone per year. Those who are cured often suffer long- term effects of treatment, which include kidney failure, cardiovascular disease, lung fibrosis and second malignancies. Therefore, our goal is to find new and better therapeutic strategies to treat this disease. Our plan is to find new potential therapeutic targets in malignant GCTs. These targets will be protein-coding or nonprotein coding genes which are dysregulated in malignant GCT cells and which are responsible for the progression of	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	These preclinical studies will set the basis for the development of new therapies to treat malignant GCTs, which particularly affect adolescents and young adults, to improve the overall cure rate and reduce the long-term side effects experienced by many survivors. the disease, or the cellular pathways they affect. After the identification of the possible targets, we will try to block the growth and spread of	

	malignant GCTs in mice by manipulating the levels of these targets with potential therapeutic agents.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use up to 4,500 mice over 5 years. We plan to use immunodeficient mice, for example athymic nude 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 animals are very closely monitored for adverse effects and are assessed on an individual and daily basis. This includes performing experiments with tumours small enough that they do not cause clinical signs. We include wherever possible the use of non-invasive techniques and, where any technique may cause more than transient harm/discomfort (than that resulting from a subcutaneous injection), employ appropriate anaesthetics and analgesics. We will look out for signs of adverse effects, e.g. social isolation, weight loss, skin sores, and any animals that reach a pre-determined limit of severity will be killed. All animals will be killed at the end of experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This project requires the use of animals because only in the context of the complete living animal we can fully understand how these cancers develop and progress. Tumours are complex and are not only formed by malignant cells but also by inflammatory cells and other cells of the immune system, as well as new blood vessels and a tissue matrix (called the 'stroma') that holds it all together. This complexity cannot be reproduced *in vitro* (i.e. in a Petri dish), hence why we need to use animals. In addition, there are no *in vitro* models to study the spread of tumours (metastasis), and it is this process that ultimately results in the death of cancer patients. *In vitro* models will be used to identify the most promising approaches and only these will be assessed in animal models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The minimal number of animals to gain statistically significant data will be used for experiments, and experimental designs will adhere to the 'Guidelines for the Welfare and use of Animals in Cancer Research' (38).

3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the least sentient species of mammal that can be used for cancer studies. Human tumours can be grafted in them without rejection and mice can be modified transgenically to spontaneously generate tumours. In a typical experiment these tumour-bearing animals receive treatments that are expected to modify the growth and spread of the cancer. The development of the cancer is then assessed, for example by measuring the tumours non-invasively with calipers. We keep suffering to a minimum, by adhering to the best practice available.

<table>
<tr><td>PROJECT 37</td><td>Molecular Imaging of Cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Imaging, cancer, instrumentation, contrast</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Our goal is to improve cancer imaging by developing more sensitive and less harmful imaging technology. At present, tumour response to chemotherapy is assessed using tumour size; if a tumour shrinks it is said to “have responded”, while if it grows, it has “not responded”. This measurement is crude, and patients often have to wait months for the size of their tumour to change. This means that they can be taking a drug for months that is not working without knowing it. Instead of measuring tumour size, we are trying to measure how rapidly the tumour cycles antioxidants and uses energy, which it uses to protect itself against the cellular damage caused by chemotherapy.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or</td><td>Cancer patients could benefit from our approach because it would enable us to determine whether their tumours are responding to therapy much earlier in the course of their treatment e.g. days to</td></tr>
</table>

animals could benefit from the project)?	weeks rather than months. Science could also be advanced because being able to image these processes in humans would help us to understand how chemotherapy drugs work and detect how cancer cells become resistant to the therapy.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse; approximately 300 over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The likely level of severity in these studies is moderate, due to the induction and treatment of tumour burden. The imaging techniques should only result in mild adverse effects. At the end of the studies, each mouse will be killed and its tumour will be removed for subsequent analysis, that will help us to validate and understand the new imaging technique.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Research alternatives, which do not involve the use of animal models, are used in the initial testing phase of all new imaging methods. However, testing of novel molecular imaging technologies for the monitoring of tumour antioxidant status and energy metabolism in living animals is also necessary. This is due to the inherent stress that is placed upon cancer cells that grow outside of the body, and the lack of supporting cells such as blood vessels that deliver oxygen and antioxidants to the tumour.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Full statistical analysis will be used to guide our studies. We will use a power analysis to calculate the minimum number of animals that will be needed to evaluate our new imaging methods.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	Mouse tumour models are important systems in which to test new therapeutic approaches and in which to develop new methods for detecting and predicting the responses of cancers to these treatments, including new non-invasive imaging

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	methods. The reason for this rapid growth in the use of mouse tumour models relates to our rapidly growing understanding of the genetic basis of cancer and our ability to manipulate the mouse, genetically and otherwise, to produce accurate models of the human disease. Environmental enrichment will be provided to improve animal welfare and promote the expression of species-appropriate behaviour and mental activities. Animal suffering is minimised by the use of anaesthetic and analgesic where necessary, but the majority of procedures are minimally invasive. No protocols are defined as severe and all moderate protocols will be continuously reviewed to ensure that any new advances that afford opportunity to reduce the severity limit still further are duly incorporated.

<table>
<tr><td>PROJECT 38</td><td>Genetics and treatment of acute lymphoblastic leukaemia</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Acute lymphoblastic leukaemia, measles virus</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td></td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The overall goals of our work are to understand more about the how of acute lymphoblastic leukaemia (ALL) first develops in adults, in particular we want to know if there are specific cells within the main leukaemia population which are responsible for initiating and maintaining the disease. Such cells may be responsible for failure of current treatments and understanding more about them is vital for progress in this area.

Related to this and stemming from our clinical understanding of the limitations of currently chemotherapy treatments, we are developing a so-called “oncolytic” (literally means lyses cancer cells) virus (vaccine strain measles) which multiplies within cancer cells and kills them, leaving normal cells unharmed, as a novel treatment for ALL. We have reached the point of beginning clinical trials in humans with this virus but there is</td></tr>
</table>

	still a lot to be learnt about how it works and aspects of its safety. Specifically we plan to: -Improve the oncolytic measles virus to be more effective and stop it being disabled by the bodies immune response -Coating the oncolytic measles virus with physical materials -Combining measles virus with existing immunosuppressive treatments to lessen the immune response -“Arming” the replicating oncolytic measles virus with specific genes to correction of known genetic defects in ALL Find out more about of leukaemia- initiating cells in ALL -To use cancer cells taken directly from patients to see which ones constitute the self renewing Leukaemia initiating cell (LIC) populations in mice -To learn more about the genetics of these cells and how they differ from the bulk of the cancer -To find out if these cells respond differently to traditional and novel treatments for ALL
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)?	Some of the data will be used directly in regulatory submissions concerning safety and efficacy to the MHRA and the Gene Therapy Advisory Committee (GTAC) to perform the clinical trial in humans. Some of the data will be used to design the best strategies for future trials, where we plan to combine existing treatments with the virus. Some of the data will be used to understand more about why current treatments fail our patients. Some of the data will simply allow us to understand more about how leukaemia develops and persists and how viruses can be used to treat cancer which

	will help our research program and that of others working in similar areas
What species and approximate numbers of animals do you expect to use over what period of time?	We will work only with mice. Most of the mice we used are immunodeficient, to allow us to develop human leukaemias in them. For understanding more about the side effects of measles therapy we have one strain of mice which are transgenic for the human receptor for measles virus, which allows the mice to become infected with the vaccine strain in a similar way to humans (ie a limited infection which doesn't cause any disease and is eradicated by the immune system but which leaves the organism subsequently immune). We expect this program of work to run for the full 5 years. We expect we may use 4-5 thousand mice over this time period. The work will form part of projects funded by various grant funding bodies (CR UK, MRC, Leukaemia and Lymphoma Research) for several PhD students and post doctoral researchers.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most of the animals used will develop leukaemia over a period of weeks or months after human leukaemia cells are injected into their tail veins on into their bone marrow (under anaesthetic). All of the mice will receive one or more injections and some will receive short anaesthetics to allow monitoring of the tumours using a special imaging camera. Progression of the leukaemia will eventually make them feel unwell – lethargic, low appetite, poor grooming, weight loss etc. They should not feel pain. The measles treatments will not affect the immundeficient mice since, the mice are not infectable by measles. The transgenic mice are infectable but the vaccine strain does not cause any illness.

	Other treatments used such as chemotherapy drugs may produce minor side effects in the tumor-bearing immunodeficient mice such as lack of appetite, fatigue and susceptibility to infections, similar to those effects observed in humans. However, it is also possible that the chemotherapy drugs might have a beneficial effect on the mice, since they are anticipated to reduce tumor burden effectively. Where mice need irradiation to allow the leukaemia to develop they can become additionally sensitive to infections and they sometimes need antibiotics in their drinking water. At the end, when any mice reach the endpoints described in our composite severity score, they are humanely killed. Often, after death their bone marrow and other organs and tissues are collected and then used for various experiments at the lab bench. Overall, the impact of our experiments on the mice is of moderate severity although some of the individual experiments and procedures are of mild severity.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Murine models are a key part of this work for two reasons: 1) The work concerns identification of cancer initiating (stem) cells; serial transplantation in mouse models is currently the only scientific definition of a stem cell 2) The murine models are needed to provide key proof-of-principle efficacy and toxcicity data to form part of submissions to funding bodies and regulatory agencies which will allow us to translate our work into the clinic.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Mice are the lowest vertebrate group in which models of cell carrier based delivery of oncolytic virus' therapy have been investigated. Our approach to numbers of mice are based on the results of numerous publications in this area, many from our group. Our experience and that of others

	has allowed us to design experiments in which the number of mice used is minimised and in which the duration and severity of the experiments are reduced as much as possible. In particular, our use in in-vivo imaging allows us to follow the mice closely a different time points rather than requiring groups of mice to be sacrificed at those time points to assess tumors
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.	<u>Immunodeficient mouse models</u> Mice are the lowest group in which pre- clinical models for oncolytic therapies in cancer have been developed. In particular, we need to use immunodeficient mice to allow human tumor engraftment. We use the least invasive method of tumor seeding possible in the least immunodeficient strain to allow the most efficient development of rhe leukaemia. For cell line, proof of principle experiments, we can use SCID mice with subcutaneous or intravenous tail vein administration. For patient-derived human leukeaemia cell engraftment, our previous work has shown that intrabone marrow injection into more more severly immunodeficient mice produces the most efficient development of leukaemia. Mice are anaesthetised during the procedure which can then proceed quickly with minimal effect on the mouse. <u>CD46 transgenic mice</u> This immunocompetent mouse strain which expresses the human measles virus receptor CD46 is required for any testing of immune response or toxicity of oncolytic measles virus since mice are not normally able to be infected by measles virus. This model is the lowest vertebrate species possible to model this situation. Other models that are traditionally used to model MV pathology are primates, which are the only animals other than humans who are readily infectable by MV. By using transgenic mice, we can avoid doing this work in primates.

<table>
<tr><td>PROJECT 39</td><td>PML and its network in tissue development and disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cancer, stem cells</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The DNA in every cell of our body is contained in a cellular component called the nucleus. Within the nucleus the DNA forms a high-order structure called chromatin, which is positioned in vicinity of subnuclear domains called promyelocytic leukaemia bodies (PML-NBs). PML-NBs are altered in many human tumours, from leukaemia to brain cancer. Similarly, alterations of chromatin have been implicated in cancer pathogenesis in multiple tissues. Both PML-NBs and chromatin processes play key roles in stem cells, which drive development and adult tissue maintenance, but can also cause cancer. We study the involvement of components of PML-NBs and chromatin in normal tissues during development and in the adult and how their alterations lead to cancer.</td></tr>
<tr><td>What are the potential benefits likely to derive from this</td><td>This work will lead to an increased understanding of fundamental processes involved in regulation of</td></tr>
</table>

project (how science could be advanced or humans or animals could benefit from the project)?	normal tissue physiology and cancer development. In turn, this may provide new ways to classify and/or treat human cancer, with potential benefits for survival and quality of life of cancer patients. Furthermore, an increased grasp of processes regulating stem cell function can aid research in the area of regenerative medicine. Ultimately, defining the basic mechanisms controlling these processes in vivo is essential to understand the pathogenesis of human disease states and could provide new tools for therapeutic intervention. Therefore, the broad implications of these studies in our view justify the use of animals.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mouse as experimental model and up to 6,500 animals will be used over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Adverse effects would be of moderate severity and will be associated mainly to alterations of tissue development and cancer pathogenesis. Animals will be humanely killed at the end of regulated procedures. For instance, we plan to inject mice that engineered to develop tumours similar to human conditions with substances with anti-tumour activity. These substances could have adverse effect, as it happens in patients, which will be closely monitored by expert staff. If adverse effects exceeds limits defined by the National Cancer Research Institute guidelines, animals will be humanely killed to avoid suffering.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	An alternative of the use of animals is to use cells derived from human normal or cancerous tissues, as also indicated by the FRAME website. Although we use this approach in the laboratory, the information gained is partial and only marginally help to define the role of genes involved in tumourigenesis. In particular, *in vitro* systems do not fully recapitulate the type of environment in which cells are inserted *in vivo*. Furthermore, when cells are cultured *in vitro* they often undergo

	dramatic alterations, thus affecting the value of *in vitro* experiments. In sum, it is difficult to model development, tissue homeostasis and tumour formation *in vitro*, and this is mainly due to the complexity of tissues such as the brain and the bone. The intact tissues with their full complement of specialised cells is the only system in which mechanisms can be fully tested and therapies be accurately evaluated. In particular, this applies to the brain, for which insufficient information exists to generate accurate computer models which can predict the complex responses of neuronal tissues. With respect to tumours, indeed some aspects of this project can and will be studied in vitro using cell lines, some of which are derived from transgenic mice or human tumours. However, to establish whether changes observed in vitro do have an impact on tumour development we need to rely on preclinical models. In this respect, extrapolating information gained from *in vitro* studies to set up new ways to treat or classify human tumours is very risky and may lead to unnecessary or even dangerous clinical studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Before embarking upon any *in vivo* experiments, hypothesis will be initially tested in *in vitro* models in primary cell culture. When *in vivo* experiments are appropriate, small pilot studies will be carried out to estimate the variability of the experimental data so appropriate statistical analysis can be used to minimize the numbers of animals required for a validated result. For all experiments, we will use the smallest possible number of animals to obtain statistically significance. When we will need to conduct experiments aimed at studying the role of a specific gene in cancer development, we will use state-of-the art imaging approaches such as MRI that will allow us to monitor the natural course of tumours growth at various stages during the development of the disease. The imaging procedure may also help minimising the number of animals that have to be kept until the endpoint of the disease is reached. To minimise use of animals as part of the experimental work, we will: • Ensure high standards of animal care,

	welfare and utilise the most appropriate breeding methods. • Ensure that colony sizes are monitored and adjusted within a formal forecasting system to meet the requirements of the research programme(s). • Ensure that breeding colonies are always kept to their minimum size so as not to over produce. Detailed breeding records will be kept enabling the selection of the most appropriate breeding stock. • Verify that before setting up or creating new transgenic lines a full search is carried out to ascertain that there is no other worldwide availability. • Ensure that Personal Licensees working on this project are appropriately trained and suitably competent to enable a high success rate to be achieved and thus minimise the number of animals used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	**Choice of species** The mouse is the only mammalian species for which genetic modification techniques are widely available and is the species that is most widely used in the international research community for studying the *in vivo* functions of genes found altered in human disease. More generally, anatomy, physiology, genetics of the mouse are similar to humans, and mice represent a very powerful system where to model human biology and disease. Therefore in order for our data to have worldwide significance, the mouse is the most appropriate species to use and will provide insights into human physiology and disease. Currently, the most effective way of investigating the function of a given gene at the level of the whole animal is through the generation of genetically altered mice (transgenics). Although it is possible to inactivate genes/proteins of key physiological/developmental processes by administration of toxins/drugs etc to whole animals, in many cases this approach suffers from lack of specificity and gives rise to more wide-ranging adverse effects that cannot be easily controlled. It also does not allow the detailed dissection of the

	complete network of genes involved in key developmental or physiological processes, unlike the use of transgenics. We have extensive experience in all the regulated procedures in this licence and will use our experience to ensure suffering is minimal. **How we will minimise suffering** The Biological Services/UCL run a comprehensive health-monitoring programme. Animal health and welfare records are maintained to include any adverse effects that may develop, particularly in genetically altered and spontaneous mutant strains. Signs consistently associated with a particular phenotype/genotype will be recorded on the respective “information sheet” in the breeding area (see above). The animals will be maintained within Biological Services/UCL under conditions where their health status can be protected as far as is reasonably practicable.

<table>
<tr><td>PROJECT 40</td><td>Models of lymphomas to identify therapeutic targets</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Lymphoma, microenvironment, targeted therapy</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Regulatory use and routine production</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Preservation of species</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Higher education or training</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td><s>Yes</s></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>1. To determine if cell signalling pathways involved in lymphoma proliferation can be inhibited using targeted therapies alone or in combination with known chemotherapeutics
2. To establish a model of the tumour microenvironment, study how it contributes to tumour proliferation
3. To modulate and target components of the microenvironment to perturb cancer proliferation and immune evasion.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>By understanding the microenvironment, we can target the components essential for cancer cell survival and proliferation. Targeted therapies provide a personalised, less toxic and more meaningful treatment course to patients. We hope to determine if certain patients with particular stratification would benefit from specific targeted therapies.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Mouse <5000 over 5 years</td></tr>
<tr><td>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</td><td>The mice have transient discomfort from restraint and needle prick from injections. Techniques such as intrafemoral injections or kidney capsule implantation where pain is possible, we will use analgesic prior to performing techniques under anaesthesia. Post-operatively, we will monitor and</td></tr>
</table>

end?	give analgesia as needed. Adverse effects expected in most animals are tumour formation, enlarged spleen, and lymph nodes. In Uncommon adverse effects are infection, maximum 20% weight loss, body condition score of 2 or less, loss of ability to ambulate, or laboured respiration. When humane endpoints are reached, animals will be culled by Schedule 1 method. Anti-cancer substances should have few adverse effects at the dose, route and schedule we use. In rare circumstances death from overdose, lethargy, or infection may occur. Adverse effects are expected to be of moderate severity. Animals are culled by Schedule 1 method or by exsanguinations. Blood, tumour and relevant tissue are harvested for further analysis
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Lymphoma mouse models are well established and have been extensively used to evaluate new therapies. We will use *in vitro* methods for optimisation but these artificial conditions do not give an accurate assessment of the effects of genetic modifications to lymphoma microenvironment or if targeted treatment at clinically relevant doses would have an effect. In order to validate specific genes as potential drug targets, it is essential to analyse their function in lymphoma development/maintenance *in vivo*. With the availability of GA mice, patient cells, and lymphoma cell lines, we will be able to mimic different types of lymphomas and recapitulate its tumour micro environment. The three-dimensional environment important for lymphoma development, complex signalling and micro-niche cannot be fully recreated in tissue culture. Mouse and human have similar immune systems. While we will test genetic manipulation of TME and lymphoma genes *in vitro* +/- treatment, only in an *in vivo* setting can we actually study its effect on immune evasion. We have extensively studied lymphoma microenvironment signalling *in vitro* and have identified 2 components that may play a role in tumour development and immune evasion. We will

	validate these targets in an *in vivo* model of lymphoma where the complexity of the TME is represented.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our group has considerable experience in developing cancer mouse models especially those involving lymphocytes. Good laboratory practise, project management, and data analysis ensures that experiments will be carried out using the least number of mice necessary to achieve significant results. Data will be shared and fed back for subsequent experiments. • We will randomise, age and sex match cohorts for treatment experiments to minimise variability • We will evaluate agents at clinically relevant doses • Pilot studies are used to determine engraftment kinetics and identify adverse effects • Only pre-screened drugs from *in vitro* experiments will be used *in vivo*. • We will always aim to use the smallest number of animals that allow us to achieve statistical significance. • We will address multiple objectives with a single experiment and gain the most information possible using the least number of mice by performing *ex vivo* experiments • *Ex vivo* samples will be frozen/fixed to help us answer future questions • Experiments will be repeated the minimum number of times to ensure reproducible and valid results. A robust model will provide reliable pre-clinical results that will ensure only the most promising treatments are developed clinically. We will publish findings in high-impact journals and present
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Our group is very familiar with cancer mouse models and have extensive experience in designing, planning, and executing mouse experiments. The mouse models we intend to use will be the most appropriate having been taken from literature and characterised/optimised by us in pilot studies. We will test our models using standard treatments to identify expected clinical effects before doing genetic manipulations. To minimise pain, suffering, distress and lasting harm, monitoring frequency will increase during

	critical periods (ie during treatment or exponential tumour growth). The user with primary responsibility will be clearly stated, competently trained, and have working knowledge of humane experimental endpoints. Body condition scoring is used along with weight and observations. We will do pilot studies for unfamiliar cells or anti-cancer agents to identify any adverse effects before doing larger experiments. *In vivo* imaging refines the monitoring of internal tumours and defines endpoints before clinical health signs are apparent

PROJECT 41	**Targeted molecular and immune therapies for cancer**	
Key Words (max. 5 words)	Cancer, melanoma, immune response, inflammation, immunotherapy	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We aim to study the pathways that lead to the development and spread of cancer and to discover and evaluate novel molecular, biological, immunological and immune cell diagnostic and therapeutic treatments, including novel antibodies. We are particularly focusing on tissue cancers such as malignant melanoma for which there are no effective therapies available. These studies will also help us understand how cancer develops and spreads in the body, and how our immune system which protects us from infections, interacts with cancer cells.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Despite a small number of circumscribed successes in the treatment of cancer, tissue malignancies represent a major group of diseases for which limited effective therapies exist. Although surgery, radiotherapy, chemotherapy and adjuvant therapy have been used, tissue cancers in their advanced	

	stages are notoriously resistant to conventional drugs and are therefore a major therapeutic challenge. Attention has recently turned to the development of novel molecular targeted and immune therapies designed to fight tissue cancers such as melanoma. Immune and immune cell therapies for cancer have been the focus of many studies because tissue tumours are known to elicit immune responses resulting in immune cell activation. A number of molecular pathways associated with cancer cell growth and a small number of tumour antigens have been targeted using molecular or immune therapies. Therapeutic antibodies are established in medical treatment against autoimmune diseases, transplant rejection and cancer. In cancer therapy, the high specificity of an antibody for its cellular target is expected to specifically target malignant cells resulting in cancer cell death by a number of signalling and immunological mechanisms. Although some of these therapeutic interventions are already approved for clinical use, their potential for the treatment of tissue cancers is far from being realised and the mechanism of action against tumour cells of many of these agents is not fully understood. The ultimate aim of our research is to understand the mechanisms of malignancy and to use this knowledge to evaluate novel targeted treatments that can help control cancer progression and metastasis and can benefit patients who suffer from cancer.
What species and approximate numbers of animals do you expect to use over what period of time?	We estimate that we may use a maximum of 7,000 rodents during the 5 year life-span of this project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will establish and study rodent models of cancer and assess the effects of various treatments on helping or preventing tumours from growing. The protocols used involve least pain, suffering or distress or lasting harm for the animals. None of the intended procedures reach beyond the moderate level of severity. Procedures reaching moderate severity arise from the induction of the disease model

	(cancer) and are necessary to evaluate the effects of potential therapies. Tumour growth is monitored very closely and animals are humanely killed at the end of the experiment or when the tumours reach a certain size.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The strategies that we are developing are designed State why you need to use for use in the diagnosis or treatment of malignant animals and why you cannot diseases, and animal experiments are crucial for use non-animal alternatives understanding the potential diagnostic use or therapeutic benefit for patients. Animal experiments are also necessary to expedite the path of these potential therapies to clinical application for the benefit of cancer patients for whom conventional treatments are not effective. We established and are implementing a range of *in vitro* strategies to study molecular, genetic and biological pathways that are involved in cancer growth, spread and progression and to derive novel antibodies by examining patient tissues. Most of our screening and evaluations of therapeutics will be conducted in culture prior to animal experiments and only the most promising treatments will be evaluated in animal studies. We wish to conduct a reduced and refined set of animal studies for the following reasons: • Due to ethical limitations of investigations in humans we depend on the use of rodents to perform significant and scientifically valid research and pre-clinical testing of therapeutics. • No *in vitro* system satisfactorily models the complex interplay of molecular, biological, immune and immune cell components and soluble factors involved in human cancer. • There is also no adequate *in vitro* model for assessment of the therapeutic value of targeting key pathways in tissue cancers. Due to the complex signalling cascades in malignant disease, animals must be used when measuring the effects of treatments. • In addition, since molecular, chemotherapeutic and biological therapeutics

	may be sequestered in different parts of the body, it is necessary to study their effects, biodistribution and retention in tumours and various organs in order to assess likely function, efficacy, toxicity and dose in advance of translating these findings into studies in patients. • In order for any targeted therapies to progress to clinical development, major insights into their pre-clinical efficacy for cancer therapy can only be derived from use of relevant animal models of local and metastatic cancers. While we shall endeavour to utilise cell culture and cultured organ models as much as possible in the development and evaluation of our therapeutic strategies, their characterisation in live animals prior to translation to the clinic, is essential and often a requirement by regulatory agencies responsible for ensuring that medicines and medical devices work, and are acceptably safe and efficacious for use in patients.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will only employ the minimum number of animals that will help us achieve meaningful results and afford statistical analysis of our data. In order to further reduce the numbers of animals we use, our experiments will be performed following the completion of pilot studies using small numbers of animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We have chosen to work on rodents as the experimental animals of choice as they have the lowest neurophysiological sensitivity while still having immune systems of comparable complexity to the human. Some of the animal work will focus on establishing tumours and assessing the effects of various treatments on helping or preventing tumours from growing. The procedures result in very little and short-lived discomfort to the animals. Tumour growth is monitored very closely and animals are humanely killed at the end of the experiment or when the tumours reach a certain size; this is done prior to procedures result in very little and short-lived discomfort to the animals. Tumour growth is monitored very closely and animals are humanely killed at the

	end of the experiment or when the tumours reach a certain size; this is done prior to any effects on the health of the animals.

<table>
<tr><td>Project 42</td><td>Regeneration and cancer in epithelial tissues</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Stem-cells; Regeneration; Tumour; Epithelial tissues</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Translational and applied research</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Preservation of species</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Higher education or training</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td><s>Yes</s></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>How cells decide to multiply themselves, or not, is still not understood. Normal tissue growth and development involves strictly controlled cell replication. When a wounded or damaged tissue regenerates, extra cell divisions occur to help rebuild the tissue. However, uncontrolled cell divisions can produce tumours. We aim to understand how cells make these decisions in tissues. We are particularly interested in an important group of genes that are well known to control these decisions in insects, but have not yet been fully examined in the mouse. Since mice are closer to humans than insects, investigating these genes in mice is important to understanding human tissue regeneration and human cancer.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or</td><td>The genes we are interested in are some of the most promising new cancer genes to be discovered in the past few years. The work on these genes has been mostly done in insects,</td></tr>
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animals could benefit from the project)?	where they are fundamentally important to controlling when are where cells multiply. Thus, investigating these genes in mice promises to advance our understanding of human tissue growth, regeneration and cancer.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice only and the project will require less than 40000 animals over the term of the licence.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will use genetic engineering to knockout several genes, or combinations of genes, in the epithelial tissues of the mouse. We will then examine the knockout mice, where tissues may grow slower than normal. We will also examine knockout mice that are regenerating their tissues after wounding, which we expect may be compromised in the knockouts compared to normal mice. Finally, we will examine tumour formation in normal versus knockout mice. Tumours will be induced by either a genetic modification or via a chemical carcinogen. We do not anticipate harms during breeding, although animals sometimes die unexpectedly during this process at a low frequency of less than 1%. Experimental knockout mice can sometimes develop unexpected symptoms, so will be carefully monitored to ensure they are healthy and pain-free. Any signs of distress will be attended to immediately to ensure the animals do not suffer. However, we do not expect any moderate or severe symptoms to occur in our skin-specific conditional knockout mice. Moderate symptoms may occur in other tissues, in which case the animals will be monitored for signs of suffering and euthanased appropriately. To examine tissue regeneration, we will need to induce small wounds of 6mm or less to test the ability of the knockout mice to heal those wounds. We expect only mild severity of suffering for these experiments. To examine tumour formation, we will need to

	induce small tumours in epithelial tissues that will not be allowed to grow to a large size that causes the animal severe distress or pain. We expect mild or moderate severity for these experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have already spent much effort investigating these genes in insects, to learn as much as possible before requiring mouse experiments. However, mouse tissues are closer to human tissues than insects, so we must perform these experiments in mice to establish the relevance of these genes to human development and disease. Our work in *Drosophila* (fruit flies) is on-going in parallel with the mouse work, and will continue to inform the mouse experiments to ensure efficient use of animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will minimise the number of animals used via use of inbred mouse strains, which reduce variability, avoiding overbreeding and keeping in mind statistical principles when designing experiments. We will also aim to maximise the amount of data we can obtain from each mouse, for example, multiple tissues will be collected from a single knockout animal and each tissue will be divided into multiple samples which are processed in different ways to yield different types of data.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The protocols we wish to use to generate and analyse knockout mice are highly developed and routinely used within our institute. We will set humane endpoints for our studies so that mice do not experience unnecessary suffering. Mice will also be monitored for signs of sufferring and euthanased at the first sign of adverse effects. We will use anaesthesia and analgesics where necessary.