Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 15

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

Project Titles and keywords

1. **Imaging and Image-Guided Therapy**
    - In-vivo, Imaging, Cancer, Mouse

2. **Functions of DNA repair genes in genome stability and cancer**
    - DNA repair, cancer, genome stability

3. **Mechanisms of brain cancer progression**
    - Brain, CNS, cancer, mouse

4. **Novel Immuno-oncology Therapies**
    - Cancer, tumour, immunotherapies

5. **Focus on the BRCA1 N-terminus in development and cancer**
    - Breast Cancer, Genetics

6. **Imaging in Drug Discovery**
    - Non-invasive, medical imaging, drug discovery

7. **Immunotherapy of cancer**
    - Immunology, Cancer, Vaccine, Therapy, Cure

8. **Novel therapies for human cancer**
    - Cancer, tumour, drug

9. **Rac and Rac regulators in development and cancer**
    - Rac, cancer initiation, metastasis

10. **Evaluation of novel treatments for prostate cancer**
    - Prostate, androgen receptor, N-terminal domain, rotenoid

11. **Using Patient Derived Xenografts to study cancer**
    - Cancer, Patient derived xenograft (PDX)

12. **Function of tetraspan complexes in carcinogenesis**
    - Tetraspan, integrin, kinase, tumourigenesis, metastasis

13. **Translational Chrono-Oncology**
    - Biological clocks, cancer, pharmacology

14. **Identifying and characterising the cellular drivers of adult acute lymphoblastic leukaemia**

- Leukaemia mouse models

15. Investigation of normal and aberrant haemopoiesis

- Haemopoiesis, cancer, bone marrow failure, graft-versus-host disease

16. Biology of Normal and Malignant Blood Cells

- Leukaemia; cord blood; fusion gene; cytokine

17. Therapeutic Antibodies for Cancer

- Monoclonal Antibody, Immune therapy, Cancer

18. In vivo assessment of biomaterials

- Cancer therapies

19. Advanced nucleic acid and small molecule therapies for cancers

- Nucleic, acid, molecule therapies cancers

20. Radiation combinations for cancer treatments

- Radiation, rodent, cancer

21. Evaluation of therapeutics for the treatment of head and neck cancer

- Head and neck, thyroid, cancer, drug discovery

22. Therapeutic strategies to improve organ quality and function in a pig model

- Transplantation, retrieval, improve organ quality

Project 1	**Imaging and Image-Guided Therapy**	
Key Words (max. 5 words)	In-vivo, Imaging, Cancer, Mouse	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	Yes	Basic research
	Yes	Translational and applied research
	No	Regulatory use and routine production
	No	Protection of the natural environment in the interests of the health or welfare of humans or animals
	No	Preservation of species
	No	Higher education or training
	No	Forensic enquiries
	No	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Imaging is widely recognized as a leading tool in the diagnosis, treatment and monitoring of a wide variety of diseases. Our work is problem-led by biologists and clinicians who require better imaging methods and we produce new and better methods to improve the study of in vivo biology, cancer development and cancer treatment.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We will deliver imaging methods that are faster, more sensitive, more specific, more tolerable or some combination of these, many of which are immediately translatable to clinical practice. The delivery of better image guided therapies will improve our understanding of the molecular and physical mechanisms of these treatments and will deliver a streamlined process for the clinical translation of better treatment protocols.	
What species and approximate numbers of	Mouse. 2000. 5 years.	

animals do you expect to use over what period of time?	Rat. 150. 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 most likely adverse effects would occur if the tumour growth occurs more rapidly than anticipated and if cumulative effects of repeated anaesthesia are found though these issues will be mitigated through daily monitoring. In these events we can detect anatomical and behavioural manifestations and advance to humane termination as appropriate. All animals will be killed at the end of the procedure.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The live animal is required because many of the phenomena we need to image only exist in the living body. Some of these, such as tissue volume or constitution are the target of the measurement whilst some of them, such as involuntary muscle jerks, the cardiac and respiratory cycles and peristalsis are confounds which corrupt the imaging process. We aim to develop imaging techniques that are insensitive to these confounds whilst maximally sensitive to the measurements required.
2. Reduction Explain how you will assure the use of minimum numbers of animals	It is well established that repeated in vivo imaging allows a significant reduction in the sample size required to achieve a particular level of statistical significance. The aim of this project is to develop techniques that enable better measurements of disease progression using imaging techniques. These improvements may offer better imaging data, better imaging data collection efficiency, and/or better animal welfare compliance. All three of these provide opportunities for minimising the number of animals required. For these works we will minimise the number of animals required by developing techniques using test objects wherever possible, and only advancing to in vivo once techniques are demonstrated to work.

	Demonstration of techniques (as opposed to, say, evaluation of drug efficacy) usually requires small numbers of animals as the measures of success tend to be binary (does the technique work or not).
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 and rat are used as these are used in the vast majority of preclinical imaging applications, and the techniques we seek to deliver will be applied in this arena. We seek to develop minimally invasive techniques that can be applied without inducing significant harm to the animal and without altering the progression of the disease to be studied. Close monitoring of animal welfare, the application of the most refined techniques (eg the application of less widely used but less harmful anaesthetics) and even the avoidance of anaesthetics altogether where this can be achieved without inducing undue stress to the animal, and the definition of humane endpoints will minimise welfare costs.

Project 2	**Functions of DNA repair genes in genome stability and cancer**	
Key Words (max. 5 words)	DNA repair, cancer, genome stability	
Expected duration of the project (yrs)		
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	DNA is a highly reactive molecule that is susceptible to damage. Fortunately, cells have evolved specialised mechanisms that are remarkably efficient in correcting specific types of DNA damage. These mechanisms play essential roles in the maintenance of genome integrity, and their deficiencies have been associated with ageing and cancer. Our aim is to improve the general understanding of mechanisms that play critical roles in the maintenance of genome integrity. We are particularly interested in discovering new pathways and characterizing novel factors implicated in this process. Our particular interest is a large family of proteins called SNF2 ATPases, which regulate a variety of activities (including modulation of chromatin structure, transcription, DNA replication, DNA repair and recombination). Importantly, many SNF2 family members have been linked to human disease and cancer. Their role in human pathologies is documented by several developmental disorders, as	

	well as by their identification as contributing factors in a variety of human cancers. Different SNF2 ATPases may play distinct roles in cancer, and whereas some act to suppress tumour formation, others promote tumourigenesis. Therefore, diversity which we observe in the cellular functions of SNF2 ATPase family is reflected in the complexity of the mechanisms that underlie connections between SNF2 ATPases and human disease. Despite a clear link between SNF2 ATPases and human disease, their as yet undefined roles in vivo leave a substantial gap in our knowledge that requires further investigation. Our aim is to overcome limitations of the in vitro systems and establish mouse model systems of specific SNF2 ATPases, as well as other genes implicated in genome stability. By establishing tumour model systems and creating genetically modified mice we hope to explore the roles of novel genes in the fundamental mechanisms that support genome stability.
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)?	Using mouse models proposed in this project will have several specific benefits. Firstly, it will provide insight into the basic mechanisms which support genome stability. These mechanisms show remarkable complexity and studying them will improve our understanding of fundamental cellular activities that play a role in cancer and human disease. Secondly, our insight into the link between genome stability and cancer may potentially suggest direct medial applications in the treatment of cancer. This is particularly relevant to the studies involving members of SNF2 ATPase family, whose roles in tumourigenesis have previously been established. Furthermore, our in vitro studies provided insight into the regulation of specific SNF2 ATPase members, which could potentially be exploited in pharmacological context. We are hoping that the proposed experiments involving mouse tumour models may suggest therapeutic strategies for tumours associated with specific SNF2 ATPases.

What species and approximate numbers of animals do you expect to use over what period of time?	Mice, 990 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?	Due to the unpredictable nature of the genetic knockout studies we aim to carry out (i.e. deletion of genes) we may produce animals which are inherently ill/abnormal in some way. Mutant mice may develop adverse phenotypes depending on their genetic modification. Possible adverse effects might include developmental defects, premature ageing, and increased incidence of spontaneous tumour development (in aged animals). Developmental defects would likely present early (for example in utero as such defects are not usually compatible with life). With the characterisation of new strains it is difficult to predict the severity of the genetic alterations and we could expect anything from complete viability (phenotypically normal mice) to embryonic lethality (lethal). However, in the case of DNA repair/genome integrity genes there does appear to be a lot of functional overlap in particular pathways, suggesting that knockout of an individual gene may not cause any overt phenotype. Breeding and maintenance of mutant mice will be covered by two protocols, one of mild and one of moderate severity. We would also like to establish an orthotropic liver cancer and xenograft models. These procedures are covered by protocols of moderate severity and expected adverse effects include development of tumours in liver and spleen which may cause weight loss/gain, abdominal distention and general symptoms of being unwell such as lethargy and poor coat condition. In addition there may be an increased risk of infection (when using immunodeficient mice), low incidence of toxicity in mice undergoing pre- and post-conditioning regimes, surgical complications arising from intra-splenic injections, weight loss during tumour growth, skin erythema, hypothermia and toxicity from drug treatment.

	We also plan to examine the DNA repair pathways in animals by administrating DNA damaging agents. The commonest side effects of which are leukopenia (loss of white blood cells) and gastrointestinal syndrome (causes anorexia, diarrhoea and loss of fluid). The animals will be frequently monitored and any animal showing the predictable signs of gastrointestinal syndrome will be humanely killed in adherence of the humane end-points. Following a procedure, animals may enter into another protocol under continued use or will be humanely killed using an appropriate method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Wherever possible we will always use non-animal alternatives. However, some important, basic biology questions can only be answered using whole organisms – such as, is gene X *essential* for life? Does gene X have a role in human disease? Does gene X affect genome stability and/or play a role in DNA repair pathways? These questions can only be answered using animals since no cell culture nor computer technology is currently sophisticated enough to replace the complexity of a whole living organism.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Even though it is not possible to replace the animals in our research, we will still extensively rely on non-animal alternatives to reduce the total animals required and to guide our research. Prior to conducting an experiment, the minimum number of animals will be calculated based on a combination of the latest published methods and in consultation with a statistician. We will continually analyse our data using appropriate statistical methods (such as power analysis) to ensure we always maintain a rigorous approach to experimental planning.
3. Refinement Explain the choice of species	Mice share similar genetics, metabolism and physiology with humans and this combined with their short generation time make them the animal model of

and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	choice for a number of different areas of biomedical research, including the genetic studies proposed here. We will constantly check the latest methods for further refinements/improvements as well be in close communication with other researchers using the same techniques to be aware of any potential adverse effects. Regardless of whether under a procedure or not all our animals will be examined daily by trained animal technicians (possibly by use of welfare scoring sheets if appropriate) and any animal displaying signs of stress or suffering such as lethargy or hunched appearance etc. will require immediate veterinary attention. Humane end points will be adhered to strictly and any animal which is in pain or discomfort that cannot be alleviated by simple treatment will be euthanized.

Project 3	**Mechanisms of brain cancer progression**	
Key Words (max. 5 words)	Brain, CNS, cancer, mouse	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	With an average life expectancy of only 14 months post diagnosis, glioblastoma (GBM) is both the most prevalent and perhaps, the most destructive of all adult human brain cancers. Notwithstanding its astounding and aggressive growth rate, the root cause of this poor clinical prognosis is arguably the highly invasive character of the malignancy. From the very earliest stages of tumor development, in addition to establishing a considerable tumor mass, the GBM also begins to infiltrate into the otherwise healthy brain tissue. These innumerable fine tendrils of tumour escape therapeutic intervention and persist, ultimately reestablishing the tumour and hastening the end stages of the disease. Our understanding of the phenomenon of brain tumour invasion is greatly limited by the lack of animal models that include this property of aggressive invasion.	
What are the potential benefits likely to derive from this project (how science could be	In order to advance our ability to treat glioma clinically, a clear understanding of the biology that governs tissue invasion on a cellular as well as a	

advanced or humans or animals could benefit from the project)?	molecular level is absolutely required. We hope that a more complete animal model of human glioma – one that includes all of the hallmarks of the human condition – will reveal new molecules that cause tumour aggressiveness. Ultimately, we hope to find new ways of treating these devastating cancers that will lead to improved outcome for patients.
What species and approximate numbers of animals do you expect to use over what period of time?	With respect to animal usage, this investigation hinges on the use of mice as models of human brain cancer. These animals will act as hosts for the disease, generating tumors that mirror the human condition. We also foresee treating these animals with several compounds aimed at hampering tumour progression. All animal use will be carried out with the intent of gaining a better understanding of the processes and biology brain cancer progression and improving patient outcome. We expect a need of approximately 4000 mice over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Some of the mice in this program of work will acquire brain tumours during their lifetime. The majority of experiments will be geared towards early stages of tumour development in these mice, however in some instances where characterisation of gene function is required, tumour progression may be accelerated. In either case, tumour size will not be permitted to exceed stipulated size limits, and morbidity will be carefully monitored, in order to minimise stress to the animals. Other adverse effects may include some neurological symptoms, which usually do not adversely affect the welfare of the affected animal. A proportion of experimental animals will also be subjected to discomfort following surgery or during injections. This will be minimised with analgesics and/or anaesthetics where appropriate. Conditional transgenics will be used where possible to limit genetic modification to the mammary tissues of the adult. This refinement will minimize detrimental impact of mutations on the animals. After an appropriate amount of time, corresponding to the extent of tumor progression, animals will be sacrificed

	and their preserved tissues will be prepared for detailed histological analyses.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Several aspects of brain cancers cannot be feasibly studied outside of the living organism. There are, as yet, no in vitro or animal-free simulations of cancer development that allow us to study the complex and multifaceted interactions of all of the interconnected cell types at play within the living organism. There are most certainly, reductionist, cell-culture models of specific aspects of tumorigenesis within the literature; however, none of these systems concern the character and function e.g. of the invading tumour. Essentially because these in vitro systems are intentionally simplified, they do not allow for the degree of complex cellular interconnectivity required for the proposed studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All aspects of this work will be supported by experiments involving cells isolated from brain tissues and tumours and maintained in culture in the laboratory. This will reduce the number of animals required. However animals are required to study tumour biology because it involves a complex interaction between multiple tissue types and the immune system, therefore analysis of tumour behaviour ultimately requires intervention in the context of the whole organism. Determining overall cohort sizes is based on previous experience with these models and validated using power analyses. The use of non-invasive imaging techniques, while part of the research and development of new diagnostic tools in their own right, will further reduce animal numbers.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to	Mice are considered the most suitable experimental animal model for brain cancer for the following reasons: there is a large body of knowledge on the physiology, histology and molecular biology of the mouse CNS; there are a wide range of existing genetically modified lines, a number of which are directly relevant and amenable to our studies; the relatively short life-cycle and high fecundity of mice is

minimise welfare costs (harms) to the animals.	advantageous for genetic studies; and mice are generally regarded as being of lower sentience compared to other mammals such as the primates.

Project 4	**Novel Immuno-oncology Therapies**	
Key Words (max. 5 words)	Cancer, tumour, immunotherapies	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
	x	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to develop novel immunotherapies for the treatment of cancer	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Cancer is one of the leading causes of mortality and morbidity worldwide. By contributing to the development of new candidate drugs, our project will benefit the patients improving their prognostic, their quality of life and reducing pain. By providing high quality services and scientific expertise, we are able to make the testing of such drugs more cost effective, more informative and reduce the need for companies to set up the models in house.	
What species and approximate numbers of animals do you expect to use over what period of time?	The estimated number of animals to be used over the duration of the project is 2000. Animals to be used are mice. They are the least sentient species which allow the objectives to be met. Up to 50% of the animals used will harmful mutants (immune-deficient 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?	Expected adverse events: bodyweight loss, lung metastases, ascite formation, adverse reaction to lead compound. Expected level of severity: Moderate. Measures taken to limit harms: frequent monitoring of disease-specific clinical signs and non-specific clinical signs for early identification of adverse events, moderate signs tolerated for no more than 24 hours, severe signs not tolerated. Humane endpoints are applied to minimise harm and include humane culling prior to the development of severe clinical signs. At the end of an experiment, all animals will be culled.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The immune system's response involves multiple systems, multiple organs and multiple cell types. The complexity of the immune responses cannot be reproduced *in vitro*. In addition, the symptoms of cancer cannot all be modelled *in vitro*. *In vitro* experiments on cell lines and *ex vivo* experiments on cell cultures will be performed. However, the limitations of these methods do not allow them to replace the use of experimental animals: there is no alternative to the use of a living animal that would allow the objectives to be met.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Power analysis will be performed to establish the total sample size required to generate meaningful data. Typically, power value will be set at 80% in order to reduce the number of animals used in the studies.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Animal suffering will be limited by choosing the sub-cutaneous ectopic models over other models whenever possible. A typical experiment will involve a systemic administration of a tumour cell line. Lead compounds will be administered prior to or from of signs of disease (prophylactic and therapeutic regimen, respectively). Animals will be monitored frequently and scored for clinical signs of disease. Blood, cells and/or tissue samples will be collected at the end of the experiment for ex vivo analyses.

<table>
<tr><td>Project 5</td><td colspan="3">Focus on the BRCA1 N-terminus in development and cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Breast Cancer Genetics</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td></td></tr>
<tr><td>Preservation of species</td><td></td><td></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></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">Scientific unknown 1. Which unclassified BRCA1 genetic variants are clinically relevant and which insignificant?

When patients with breast cancer in their family undergo a gene test sometimes small alterations in the front of the BRCA1 gene are found. However while many changes have been found very few are known to be related to cancer predisposition. Because we don’t know what the front region of BRCA1 does in cells we cannot identify which change is dangerous and which of no consequence. Patients are therefore not currently helped by the genetic result.

This project will address which small changes in the front of the BRCA1 gene can cause cancer. It will thus specifically inform certain patients and their</td></tr>
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	families about their risk of cancer development. Scientific unknown 2. How does the 'front' of BRCA1 protect again cancer development? A few gene changes in the 'front' of BRCA1 have been found in large, independent families to be in those women who have developed disease, and are thought to be pathogenic. Some changes in the beginning of the BRCA1 gene have been found to correlate with breast and ovarian cancer . Recent advances in research have suggested that the front portion of BRCA1 does something different to the rest –but as yet scientists are unsure what. Breeding our animal model, which has a change in the front of its BRCA1 gene, with animals that have different gene defects will establish which other genes are needed for (or protect from) cancer development. This understanding will be a significant step towards establishing what this portion of BRCA1 does in the prevention of breast cancer. Scientific unknown 3: Do faults in the front of the BRCA1 gene render tumours sensitive to certain types of anti-cancer therapies. Some cancers can be killed more effectively by certain drugs. For example we know that cancers that entirely lack BRCA1 are sensitive to PARP-inhibitors (a group of pharmacological inhibitors of the enzyme poly ADP ribose polymerase (PARP)). However recent findings that the beginning of the BRCA1 gene may not work in the same way as the rest has led to doubts about how to best treat cancers with changes in this region. An important part of the research will be to test agents to find out which is the best to apply to these types of cancers. Importantly the sensitivity of the tumours to drugs will also inform us about which cellular pathway is disrupted, answering scientific question 2.
What are the potential benefits likely to derive from this project (how science could be	It will inform how cancer forms following inheritance of a BRCA1 gene change, with potential implications for understanding cancer driven by

advanced or humans or animals could benefit from the project)?	such changes and also for cancer that is not directly caused by a BRCA1 genetic change. This research will give clinical professionals and certain patients and their families more information whether certain BRCA1 gene changes increase the risk of cancer or not. It will also give information on what drugs are best for treating this type cancer and potentially others (for example with 'silenced' BRCA1) It could lead to new cancer therapies, or a change in the current primary treatment choice for certain cancers. This could lead to new cancer therapies, or a definition of the most useful class of cancer with changes in the beginning of the BRCA1 gene. By defining the molecular pathways involved in tumour suppression by the Brca1 _N-terminal region this project could lay the foundations for screening and treatments of sporadic breast cancer and possibly other cancer types.
What species and approximate numbers of animals do you expect to use over what period of time?	We will be using approximately 5000 mice to breed over the whole project, and 2000 mice for tumour testing.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	All mice have a small piece of ear or tail taken to use for identification and to tests which gene changes they have. They may also have blood taken for tests. These mice may develop tumours because of the gene changes, and this may cause stress and pain to the mouse. The mice may be treated with cancer therapies or labelling markers through their food and water, or through an implant or through an injection into their skin, their underbelly, or their tail. The mice may also be injected with tumour cells into their breast area or into their skin. All of these procedures cause minimal pain and stress, and do not need pain killers or for the animals to be unconscious. It may be necessary to put a dose rate pump or drug pellet under their skin but all these procedures will be carried out with pain killers and whilst the mice are

	unconscious. Anything carried out on an animal will be done by a fully trained person and with their animals' welfare as the priority. If any of the mice are suffering excessively as a result of the tumours they bear or the agents they have been treated with they will be humanely killed through an approved Schedule 1 method. Some animals may receive doses of Irradiation. These are low doses that have not previously been reported to have any adverse effect. The animals will be closely monitored after the dosing to be sure of their condition. Some animals may be treated with chemicals that are known to cause breaks in DNA. Some of these have adverse effects. They will be given at doses that are known to be tolerated, based on previous work (published in the literature). Nevertheless side effects are possible which include : bruising or bleeding, anaemia, nausea, loss of appetite, bladder irritation, lethargy. Doxorubicin may also cause discoloured urine (pink-red, for 48 hours) skin darkening (excess production of pigment), and dry mouth. These animals will be closely monitored and with reference to a detailed body condition scoring chart and behavioural ques, those showing signs of distress will be killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The mouse has been chosen because of the wealth of other gene changes available (other models) and because we have demonstrated similarity between mouse and human cancer susceptibility through Brca1 gene change. Mice have been used for breast cancer as they have shown similar breast cancer development to humans. Using this mouse work alongside the use of molecular and cellular techniques we hope to predict which gene changes are likely to pose a risk of cancer.
2. Reduction Explain how you will assure	Statistical analysis using previous knowledge of tumour incidence will be used to ensure the correct number of animals used to give meaningful

the use of minimum numbers of animals	answers in experiments. Where possible research during this project will use cells and cell lines, for example cellular features that are close proxy to cancer-state. Similarly cells from embryos that result from the breeding of two genetically altered mouse strains will be altered so that they can grow in the laboratory indefinitely. This way the maximum information can be gleaned from the cells from animals instead of using more mice. We will also be taking mouse cells to make cell lines for further study, rather than more 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.	Protocol 1 involves the breeding of the mice and will involve minimal suffering –a negative result is embryonic lethality at an early stage prior to the development of nerves and a positive result is expected to be normal development to term. Protocol 2 is to closely observe the mice with two copies of a Brca1 gene change (once viable). We anticipate these animals will be protected from tumour development, however if we are wrong they will develop tumours. Our protocol involves frequent and careful monitoring of these animals to ensure they are killed before suffering occurs. This protocol also involves the transplant of any tumours that may occur into new animals followed by drug administration. In the event of signs of pain or distress due to drug intolerance, the dose will be reduced in treated animals or in the case of significant weight loss the mouse will be killed. Protocol 3 involves the subcutaneous transplantation of tumour cells and the treatment of any tumours that develop. Measuring the tumour development with a medical imager will allow us to use fewer animals and earlier end points. Frequent and careful monitoring of these animals to ensure they are killed before suffering occurs similarly signs of drug intolerance will be responded to swiftly.

<table>
<tr><td>Project 6</td><td>Imaging in Drug Discovery</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Non-invasive, medical imaging, drug discovery</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>Yes</td><td></td></tr>
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<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>Medical imaging techniques are enormously important in human medicine (e.g. magnetic resonance imaging (MRI) scan, computed tomography (CT) scan, positron emission tomography (PET) scan). They are also extremely valuable in animal research, where they can provide better data with fewer animals and less suffering. Nowadays, the measurements we make in medical imaging are often called “biomarkers”. Such imaging biomarkers are often used in drug research development, to see, in an animal or in a human patient, if the drug is working. The aim of this licence is to improve imaging biomarkers and methodologies to give faster and more accurate readouts.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be</td><td>The imaging methods and techniques will be used to assess novel drug safety and efficacy in drug development, including new cancer drugs. Data</td></tr>
</table>

advanced or humans or animals could benefit from the project)?	generated will be used to help provide better scanning protocols in humans, when assessing our novel drug compounds..
What species and approximate numbers of animals do you expect to use over what period of time?	It is estimated that no more than 2400 mice and 600 rats will be used during the lifetime of this licence. Numbers of animals used will be carefully calculated in order to use the minimum required in order to meet the scientific objective.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Animals are typically anaesthetised for imaging procedures. Animals may undergo one or multiple imaging procedures and may also be exposed to drug treatments and tumour induction. There are two mild severity protocols and one moderate severity protocol. All animals are carefully monitored by trained staff and housed in modern facilities. At the end of the study animals will be humanely euthanised.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In most instances imaging methods will initially be developed and validated using phantoms (a specially designed object used for scanning) and/or cadavers before progressing onto animal imaging studies. Non-animal alternatives are used in the identification and selection of imaging agents and drug compounds and generally include measurements of the likely effect of the agent on the target cells. Activity in particular cell types however, cannot predict the likely *in vivo* activity given the complexity of issues such as bioavailability and metabolism and therefore the whole animal is needed for the studies proposed in this licence.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Prior to imaging studies being performed, expert imaging scientists review whether an imaging biomarker will provide the required data and whether this is the most suitable approach. If these criteria are not met, then the imaging study will not

	be carried out. For each individual experiment statistical analyses will be performed to determine the number of animals needed. Imaging studies can allow each animal to act as its own control (the same as patient imaging studies) and allows paired comparisons. This increases the statistical power of experiments and decreases the number of animals needed compared to terminal studies.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Naïve and tumour bearing mice and rats will be used under this licence. Where ever possible non disease model animals will be used to develop, evaluate and validate imaging methodologies or techniques. Typically the imaging methods under this licence are carried out under general anaesthesia. Wherever possible sampling, for example, blood sampling to measure circulating glucose levels, are carried out whilst the animals are still anaesthetised to minimise suffering. In addition, anaesthetic and imaging time points are carefully considered and kept to the minimum possible whilst still achieving the scientific aim of the study.

<table>
<tr><td>Project Title 7</td><td>IMMUNOTHERAPY OF CANCER</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Immunology, Cancer, Vaccine, Therapy, Cure</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></tr>
<tr><td>Translational and applied research</td><td>Yes</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</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>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 overall aims of this project are:

1) To develop new immunotherapeutic strategies, that is to develop new protocols that rely on the patient’s immune system to prevent or treat cancers.
2) To minimize/prevent adverse effects of immunotherapeutic strategies, while maintaining their anti-cancer effect</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 results will increase our understanding of how cancer interacts with the immune system and thus, we will be able to optimize the experimental strategies, in order to efficiently treat cancer. In addition, we will obtain data regarding the safety and efficacy of these strategies that are required to initiate clinical trials in cancer patients.

Findings will also be published in widely read journals to spread the knowledge to the scientific community.</td></tr>
</table>

What species and approximate numbers of animals do you expect to use over what period of time?	We have confined our experiments to mice. It is estimated that 4300 mice will be used during the 5 years 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 plan to inject tumour cells in mice in order to induce cancer, with the aim to test if our therapeutic strategies can control tumour growth and if they can be well tolerated. Anticipated adverse effects, caused by cancer and/or anti-cancer treatment, include loss of appetite, weight loss hunching, piloerection, lethargy, difficulty moving, difficulty breathing, skin rash, pallor diarrhoea. In all cases, the effects are not expected to be more than moderate in severity and mice developing adverse effects during the course of the study will be humanely killed. All remaining animals will be killed at the end of the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Simple immune and tumour cell interactions can be adequately investigated in the laboratory. However, it is impossible to reproduce the more complex interactions on dishes in the laboratory so we need to use living animals to be representative of the complex interactions that occur between body systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To assure use of minimum animal numbers, small-scale preliminary experiments will be carried out to determine the minimal numbers of mice required for statistically significant and reliable results. This will help minimise the total number of experiments required.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs	All our animal experiments will be performed using mice, some of which may be genetically modified. There is a lot of published literature already available on mouse cancer models that will help guide our work. Results obtained using mouse cancer models are already accepted as being indicative of responses in humans. As already explained small-scale preliminary experiments will reduce the number of animals required. In addition, only experiments that are likely to be relevant to

(harms) to the animals.	use in human clinical studies will be employed. Moreover, tumour growth will be closely monitored using technology applied to imaging patients with cancer, thus ensuring that mice will be humanely killed before they develop discomfort.

<table>
<tr><td>Project 8</td><td>Novel therapies for human cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cancer, tumour, drug</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></td><td>No</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>There is an urgent clinical need for more effective anticancer agents for some cancer types with very poor survival rates, such as advanced pancreatic, prostate and breast cancers. The aim of this project is to test the pharmacology and anti-tumour activity of experimental agents (alone or in combination) in mouse models of cancer. The objectives are to determine the pharmacokinetics (PK, what the body does to the drug) and pharmacodynamics (PD, what the drug does to the body) for each drug and then use this information to design studies to test whether the drug or drug combination has anticancer effects. In addition, we aim to investigate the mechanisms of drug resistance for specific drugs in certain cancers such as pancreatic cancer, to identify new drugs that can be used in combination to increase response.</td></tr>
</table>

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 PK and PD data will enable collaborating drug discovery groups to direct their medicinal chemistry effort into designing compounds with better drug-like properties. Then identification of drugs and combinations with anti-tumour activity, coupled with data on drug resistance mechanisms, will permit go/no go decisions about progressing the drug(s) into clinical trials in man. The data generated herein will assist with the design of the clinical trial protocols (such as identifying the optimal dose schedule and biomarkers for detection of early response). In the longer term these studies may contribute to patient benefit by identifying more effective cancer treatments. In the shorter term it will advance the scientific literature on cancer drugs.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse. Maximum 3,900 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?	Approximately 50% of the mice will be used for breeding to produce mice with the correct genes for use in experiments. Those breeding mice will experience only mild severity as no significant adverse effects are expected. The majority of the other 50% of the mice will either develop a tumour, or be implanted with a tumour, and have drugs administered. The majority (>90%) of those will experience up to moderate severity, due to the combination of tumour burden, transient discomfort from drug dosing, periods of general anaesthesia (e.g. for imaging) and some adverse side effects of the drugs. The mice will be killed humanely at the end of the experiment, with blood and tissues taken for subsequent analysis to provide data on the pharmacological effects of the drugs. Four of the protocols have a substantial severity limit because while we aim to limit all mice to a moderate level of severity, we anticipate that a small number of mice (<1%) may succumb rapidly to adverse effects of the tumour or may exhibit an unexpectedly severe

	and acute reaction to a drug.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Studies of the effects of drugs on the body and on the whole tumour in its complex microenvironment have to be performed in live animals. However we do use sophisticated cell co-culture assays with mixtures of cell types to mirror the tumour microenvironment and to better predict efficacy of drugs in mice, in order to reduce the number of mice used.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Power calculations will be used to determine how many mice are required for studies to show statistical and biological significance
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 majority of animal models of cancer have been developed in mice, and there is a longstanding literature on cancer biology and pharmacology based on mice, which provides the background to our studies. We will choose the specific mouse cancer models that show biology most similar to the human cancer subtype of interest for each study. Animal suffering will be minimised by the use of anaesthesia and analgesia where appropriate, and environmental enrichment will be provided to promote the expression of species-appropriate behaviour. To avoid the risk of many mice experiencing substantial toxicity from novel drugs, pilot studies will be performed in small numbers of mice to identify a tolerated dose for subsequent studies.

Project 9	**Rac and Rac regulators in development and cancer**	
Key Words (max. 5 words)	RAC, CANCER INITIATION, METASTASIS,	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	Yes	Basic research
	Yes	Translational and applied research
	No	Regulatory use and routine production
	No	Protection of the natural environment in the interests of the health or welfare of humans or animals
	No	Preservation of species
	No	Higher education or training
	No	Forensic enquiries
	No	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Rac signalling regulates a diverse network of many cellular processes including the cell cycle, cell adhesion and cell migration. If these processes become disrupted due to the aberrant signalling of the Rac pathway, it can result in uncontrolled cell division and motility leading to cancer initiation and spread (metastasis). However, some aspects of the mechanisms governing Rac signalling still remain poorly understood. Therefore, we aim to address the roles of Rac and Rac regulatory proteins in: 1) Development - specifically in tissues where Rac has been previously implicated in tumour formation 2) Tumour initiation, progression and metastasis.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The expected benefits of the work can be summarised as follows: 1) A deeper knowledge of the role of Rac and its regulatory partners during development 2) Contribute to the understanding of tumour initiation, progression and metastasis mechanisms 3) In integrating the knowledge gained from both	

	of the above, identification of key therapeutic targets for future hypothesis driven chemotherapeutic intervention 4) Publication in peer-reviewed journals to share the work with the wider scientific community
What species and approximate numbers of animals do you expect to use over what period of time?	During the next 5 years, approximately 5500 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?	Mice will be bred to generate specific combinations of mutations of both mild and moderate severity. Although the majority of mice will not show any adverse effects, phenotypes may be exacerbated upon administration of substances with transgene altering properties. Other mice may per se be expected to develop spontaneous tumours. As mice are expected to eventually develop tumours, they will be closely monitored and culled at signs of persistent discomfort or if the tumour size reaches the recommended guidelines. All animals will be monitored closely and will be humanely culled if unexpected ill health occurs, if severity limits are approached or if scientific objectives have been attained.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	While valuable studies of human cancer are performed using tumour material and cell lines derived from both mice and human samples, the mechanistic understanding of cancer pathogenesis requires use of living animals. In particular, cancer development and spread involves a plethora of interactions between cancer cells and their surrounding host and their behaviour is governed by multiple signals originating from both their immediate neighbours and from distant tissues. The study of cells in culture (in vitro) provides us with clues on the mechanisms of cellular processes in a simple and valuable context, which allows the establishment of hypotheses regarding the function of cells in a living animal. However, these systems do not recapitulate the complex cellular interactions

	described above. Therefore, transgenic mouse models have been engineered to develop cancers, which accurately mimic their human counterparts, and have potential applications to test the effectiveness of novel cancer therapeutics. This cannot be replaced by in vitro studies or indeed even in different in-vivo models such as zebrafish or insects which remain far less complex than their murine counterparts.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Use of in vitro methods limits the number of animals required for the in vivo investigation stage. For transgenic models, efficient breeding strategy will minimise the number of mice used to obtain the desired genotype. The proposed experimental designs and methods of analysis of the results are always in agreement with statistical guidelines to provide meaningful data minimizing the number of animals used in each experiment. Pilot studies will be performed, in which a small number of animals per group are used for genotype comparisons. Depending on the results obtained from pilot studies, larger cohort studies will be performed to determine if the observed difference is statistically significant. When possible, tumour development will be followed up using whole body scanning as it allows a reduction of 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.	Mouse models that are in current use faithfully recapitulate the human disease. Moreover, the mouse genome shares 98% homology with human genome. Continuous improvement in husbandry and procedures will minimise actual or potential pain, suffering, distress or lasting harm and/or improve animal welfare in situations where the use of animals is unavoidable. Appropriate anaesthetic and analgesic regimes will be used as well as appropriate humane methods of culling within animal facility. No visualization of procedures by other animals is ensured and transport arrangements between facilities is made in appropriate containers.

<table>
<tr><td>Project 10</td><td>Evaluation of novel treatments for prostate cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Prostate, androgen receptor, N-terminal domain, rotenoid</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>2</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>The objectives are to determine whether two classes of compounds, synthesized by collaborators, are effective therapeutic agents for prostate cancer. The androgen receptor plays a very important part in the growth and progression of prostate cancer to drug-resistant disease, and currently used drugs target one part of it. However this part is very frequently modified, so that resistance to these drugs develops. Our agent(s) are specifically designed to target another more stable part of the molecule, so we think that resistance is less likely to occur. We need to compare the efficacy of our agents with the currently used drugs. We will use tumours grown from cell lines specially engineered to allow this to be tested. The second class of drugs targets a major metabolic difference between cells in the</td></tr>
</table>

	normal prostate and its tumours, part of the energy-generating mechanism. This is a novel target and we want to find out whether attacking it will have a sufficiently specific outcome to recommend a programme for a new type of drug development.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The early benefits will be in terms of additional biological knowledge about the mode of action of the androgen receptor and how it interacts with drugs designed to stop it from acting. If the new drug (or a derivative) is successful, and is shown to work better in animal models than the currently used drugs, it may progress to clinical development. If successful, it could prolong the lives of the 9,000 men who develop hormone-refractory (resistant) prostate cancer every year in the UK. Work on the second class of drugs is at an earlier stage, but will give valuable information to scientist on whether the particular target is worthwhile to work on, and if any of the drugs in this class work well in animal models, they could potentially be developed into valuable drugs against prostate cancer in the future.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice, about 1400 over a 2 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 adverse effects are: those associated with treatment with anti-cancer drugs, eg gut problems or anaemia etc, but these will be minimised by careful determination of well tolerated doses. The second series of drugs has been specifically developed to avoid the known neurotoxicity associated with similar molecules, but we can only test this in animals. Many animals will have subcutaneous tumours, which may sometimes ulcerate. The maximum level of severity for any animal is moderate.
Application of the 3Rs	
1. Replacement	We need to use animals because we have progressed as far as possible with in vitro work on

State why you need to use animals and why you cannot use non-animal alternatives	cells in culture and now need to examine the behaviour of the drugs in a complete mammalian system. The complexity of a solid tumour cannot be fully reproduced on a bench, nor the variations in physiological parameters. The ability of a drug to get into the brain or nervous tissue cannot be adequately modelled outside an animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will acquire the immunosuppressed animals we need from other projects authorised to supply them rather then breeding our own for a relatively short term project. We will use power calculations to determine the smallest number of animals we can use while still deriving statistically and biologically sounds data from every 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 will use mice because a very good strain of immunosuppressed mice is available, and this allows the human prostate tumour cells (with the particular properties we need) to grow in the model system which is the closest possible to man. This will give us the best possible chance to get data which will tell us exactly what would happen in human patients.

<table>
<tr><td>Project 11</td><td>Using Patient Derived Xenografts to study cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cancer, Patient derived xenograft (PDX)</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The main aim of our work under this licence is to devise care and treatments that are more representative to the patient by using PDX mouse models of cancer (specifically blood, breast and pancreatic). We hope to achieve certain goals under this aim;
3. Establishing good working models of our PDX samples, involving the correct passaging and maintenance of the PDX.
4. Identifying genes that infer resistance of triple negative breast cancer to chemotherapy.
5. Identify genes that can predict a response to the PLK1 (or other checkpoint inhibitors) and how we can enhance the response
6. Test and validate other genetic targets resulting from our in vitro screens on pancreatic cancer.</td></tr>
</table>

	7. Test and validate other genetic targets resulting from our in vitro screens on breast cancer. 8. Identify genetic signals between cancer associated fibroblasts and pancreatic cancer and how these correspond to gemcitabine resistance. 9. Identify lncRNAs that are involved in human leukaemia
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We anticipate finding a selection of genes that will further our understanding of individual cancers and cancers as a whole. We also hope to find ways to target specific cancer subtypes and identify why some are more resistant to therapies than others. Some of these findings could ultimately lead to novel therapies for cancer treatment and/or preventions. In addition we will publish our findings in peer reviewed journals, thereby sharing with the scientific community so that our data and methods can help others working on similar projects.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice 7500 over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Due to the nature of the projects the mice will undergo surgical procedures and/or develop tumours/malignancies, however these are anticipated to cause only mild discomfort, and pain relief will be given where necessary. Their progress will be closely monitored to ensure they are not suffering beyond expected. All animals will be humanly culled at the end of the experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	The ability to truly study a patient's cancer can only be done in two ways, using the cancer as it is in the patient or taking the cancer and

use non-animal alternatives	growing it on another organism. Maintaining a cancer in vitro is not always possible, based on the nature of the cancer itself, and in addition, it is well known that the cancers used as cell lines today have under gone considerable changes since they were first taken from the patient.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Where possible we will do non-essential and preliminary studies on cell culture systems of the PDX, to establish working protocols, and initial data collection, so that we can develop strategic experimental plans for the use of in vivo PDX systems. We will assess viability of frozen PDX samples that we will sample from each passage and if these prove to be a true representation we will look to using these as an alternative to continued mouse passaging
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We have chosen to do this work in mice. These are best suited for this project as they capture human disease relatively faithfully. In addition there are a large number of models available to us. We will minimise the animal suffering by monitoring the growth of the tumour and ensure it does not extend beyond regulated guidelines. The surgeries will be performed under published best practise guidelines, or where we have modified these to reduce suffering further. Preliminary studies show that the mice recover very well after surgery and are fully active upon waking.

<table>
<tr><td>Project 12</td><td>Function of tetraspan complexes in carcinogenesis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Tetraspan, integrin, kinase, tumourigenesis, metastasis</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>
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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 aim of the project is to reveal key elements of a specific molecular network that controls tumour growth and spread as metastatic progression. A subsequent goal is to target specific components of the network with newly designed inhibitory reagents.
Our main overall objective is to identify key components of a carcinogenic pathway which is centred around the molecule called CD151, which can be subsequently used as both response predictors to current anti-cancer drugs and new druggable targets.
Specific questions:
1) How do proteins that are functionally linked to CD151 affect its carcinogenic function?
2) How do the host immune responses affect</td></tr>
</table>

	carcinogenesis potentiated by the CD151-centred carcinogenic signalling network? 3) *How does the presence of various components of the CD151-centred carcinogenic signalling network affect tumour responses to current and new therapeutic reagents?*
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We anticipate that the proposed work will provide a definitive conclusion on the involvement of the proteins functionally linked with the components of tetraspan complexes in the tumour growth process. It will also establish new in vivo model systems for more detailed examination of the role of these proteins in tumour growth. In the longer term establishing these models is absolutely essential for future work testing a new class of drugs, the design of which is based on the detailed structural analysis of tetraspan complexes. We hope these novel studies will lead to more rationally designed, more effective cancer therapies.
What species and approximate numbers of animals do you expect to use over what period of time?	In our experiments we are planning to use mice. Approximate number of animals: 7200 over 5years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The level of severity for all proposed work is moderate. We expect animals to develop tumours. These animals may experience mild degrees of weight loss, reduced activity, abdominal distension, jaundice or piloerection prior to reaching their humane endpoints . Some of the animals may also develop early signs of anemia in concert with a swollen abdomen. In rare cases animals may develop early signs of infection following surgical procedures or, after systemic hormonal treatment they may experience early stages skin inflammation, thickening or loss, or altered pigmentation, benign dermal cysts, urinary tract infections or skin erosions. Appropriate moderate humane endpoints have been set within the licence, and at the end of the work animals will humanely

	killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are planning to perform extensive in vitro experiments before planned in vivo work. Although these experiments will narrow down the number of possible targets for testing in vivo, we will not be able to avoid animal experiments completely. Firstly, it is impossible to faithfully reproduce the tumour microenvironment in vitro, a key contributor to carcinogenesis. Secondly, only animal experiments allow general toxicity evaluation for newly developed anti cancer agents.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In all our experiments the number of animals per experimental condition for each cohort is based on both our previous work and work published by others in which similar type of measurements were carried out. In the majority of these experiments data distribution is expected to follow a normal distribution and therefore application of parametric statistical tests like ANOVA will be appropriate. Should the situation require a more complex statistical analysis, we will approach the Statistical Service Unit for advice. Pilot experiments will be performed to establish an active concentration of new therapeutics that do not cause general toxicity, and to determine the earliest end points. The proposed number of animals is calculated based on the similar type of experiments performed by other researches and in consultation with statistical advisors within cancer sciences.
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.	Wherever possible we will use non-invasive techniques to evaluate tumour growth and metastatic disease spread. This will allow us to assess the dynamics of cancer growth and progression in a single experiment, and, consequently, make an informed decision on the duration of the experiments. In the experiments testing anti-tumour effects of various compounds we will either use a reported dose which is known to have anti-tumour effects

	and cause minimal general toxicity or, when testing new reagents, perform pilot studies to assess the maximum tolerable dose. We will work in accordance with the recommendations described in “Guidelines for the welfare and use of animals in cancer research” (Workman, P. et al. (2010), BJC 102, 1555-57).

Project 13	**Translational Chrono-Oncology**	
Key Words (max. 5 words)	biological clocks, cancer, pharmacology	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Cancer chronotherapy has already shown great promise in experimental models and some results in the clinic. The underlying mechanisms, however, are only partly understood, and clinical benefits from a personalized approach are largely unexplored.	
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 chronotherapy has the potential to make current treatment options for cancer more effective and to reduce side effects at the same time. An understanding of the underlying mechanisms will also foster new treatment approaches. Chronotherapy will further improve the development of new potential anticancer drugs, and better help translate the experimental finding into the clinic.	
What species and approximate numbers of animals do you expect to use over what period of time?	Mice (including several transgenic mouse models) and rats (7’OOO and 1 ‘000) will be used over a period of 5 years.	
In the context of what you propose to do to the animals,	We expect only moderate severity in a sub-population of animals. Possible adverse effects that are likely to	

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	occur only in a portion of animals include cancer cachexia, tumour related suffering, treatment adverse events, food deprivation, surgery related complications and 'jet-lag induced" unwell-being. At the end of experiments, animals will be euthanized.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The Circadian Timing System (CTS) is a multicomponent dynamic system that regulates most biological functions over the 24 hours. Cancer processes involve cancer cells, but also many interactions with multiple host functions. Treatment effects also involve dynamic drug disposition processes in multiple physiological compartments including host and tumour tissues. Therefore, there is no alternative to the use of experimental in vivo models. However, we do utilize in vitro cellular models of basic clock function and have established in vitro bioluminescence reporter technologies to assay for example the impact of drug molecules on the circadian oscillator per Se. This can lead to better predictions of what might happen in vivo and will also be used as input for the in silico models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of mice in each experiment will be computed according to both prior experience and the expected magnitude of difference in main endpoint, and in vitro-in silico approaches will be pursued and further extended, so as to reduce and refine animal experimentation. The use of continuous physiological and molecular monitoring in individual mice enables the gathering of dense and precise longitudinal data, which thus help minimize noise (reduce group sizes) and reduce group numbers, e.g., instead of having to cull subgroups of animals to gather those information, e.g. gene expression in tissue samples at defined times in the course of the experiment. Finally, in mild severity experiments, numbers could further be reduced because animals could be used in cross over designs (e.g., be there own controls) in case of verified return to baseline condition between two tested sequences. Especially, the use of in vivo bioluminescence recording in freely moving animals

	has the potential to reduce the number of animals used in circadian and cancer experiments by manifold. Instead of culling animals for tissue/tumor samples every four hours for 6 times, we can obtain non invasive data in a single animal for multiple 24-h cycles. The combination of these in vivo circadian biomarkers approaches combined with systems chronopharmacology models which arise from in vitro-in silico studies will further support a reduction in the number of mice required in order to demonstrate CTS-based strategies for preventing cancer, for halting cancer progression, or for improving treatment effects.
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 (Mus musculus) and Rats (Rattus noivegicus) will be used. They both constitute reference models for chronobiology, chronopharmacology and chronotherapy investigations. Online, automated and stress-free behavioural, physiological and/or molecular monitoring contribute not only to the reduction of the number of experimental mice, as a result of the better precision in parameter quantification from dense longitudinal time series but also helps to minimize suffering for the animals. For example, we will be able to shorten experiments to absolutely necessary period the animal remains in an experiment, or can estimate quality of baseline data before the start of an experimental procedure because the relevant outcome measures are available in real-time online. Furthermore, suffering of the animals can be more fairly judged because data for food intake/activity/body temperature are excellent biomarkers and can even function as indicators for alternative endpoints.

Project 14	**Identifying and characterising the cellular drivers of adult acute lymphoblastic leukaemia**	
Key Words (max. 5 words)	Leukaemia mouse models	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	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)	Acute Lyrnphoblastic Leukaemia(ALL) in adults is an incurable disease for the vast majority (60%) of patients. The overall aim of this research is to investigate why adults with ALL relapse so often. We think there are “leukaemia initiating cells (LIC)” which are the “seed” from which leukaemia grows that can persist in patients after treatment and cause cancers to come back. We want to explore the nature of these special LIC cells. If we understand how these cells work we hope to be able to design better treatments for patients. We will also investigate whether there are LIC cells which lie asleep and this is also known as dormant. By lying asleep these LIC cells can resist treatments such as chemotherapy and cause the cancer to come back. If we can work out how to switch these sleeping dormant cells into being awake we may be able to improve treatment success in patients.	

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	By understanding in depth the behaviours of Leukaemia initiating cells we hope to better understand why adults with ALL relapse and help guide the design of better treatments.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice will be the only species of animal used in this research plan. Their nervous system is less sensitive than higher mammals, but still allow us to understand human biology. All experiments will be designed to employ the minimum number of animals that would achieve a meaningful result. Over 5 years we estimate to use approximately 2000 mice. Animal suffering will be kept to an absolute minimum by the application of good experimental technique and better practice including careful monitoring.
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 basic model for our studies requires leukaemia development in the mouse following injection of human leukaemia cells. We have developed less invasive methods for monitoring of leukaernia development such as direct bone marrow sampling under anaesthesia and light emitting leukaemia cells to track the progress of leukaemia development prior to the onset of disease symptoms. This will allow us to achieve our study aims with minimal animal suffering as we can intervene before the mouse develops other symptoms relating to leukaemia. Furthermore, we will implement measures that will maintain the wellbeing of our mice by administering antibiotics and avoiding uneccessary handling. Based on our vast experience with these models the maximum severity expected is moderate. We aim to employ wherever possible approaches to minimise any discomfort experienced by the animals by using anaesthesia and pain relief.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We currently do not know which cells within a tumour constitute the leukaemia initiating cell. To move the work forward we need to evaluate subsets of tumour cells for characteristics that are unique to a leukaemia stem cell. One of the defining characteristics of a

	leukaemia stem cell is the ability to sustain leukaemia growth long-term in a mouse, that is to make at least one copy of itself upon cell division. Cell lines are not appropriate for the study of leukaemia stem cell populations or dormancy because they continually grow and multiply unlike human leukaemia.. These points taken together indicate the requirement of animal studies for the study of a leukaemia stem cell population. Furthermore, leukaemia cells taken directly from patients do not survive or grow in a test tube making this approach unfeasible for the study of leukaemia initiation, this is why we need to use mice for these studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will design experiments to use the minimum number of mice while allowing statistical analysis of the results. Such planning will minimise the number of repeat experiments required to confirm results. Where possible we will optimise experiments using alternative models such as drug dosing on cell lines.
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 need to keep the suffering of the mice used for our experiments to a minimum is always taken into account when planning the experiments which are all undertaken with due regard to the 3R's of reduction, refinement and replacement and the Home Office guidance, and other national guidelines. In each experiment mice will be observed very closely for adverse effects and steps will be taken to minimize pain or discomfort. Mice will be housed in cages with environmental enrichment and will be subject to good, sympathetic and humane animal handling, injection and blood sampling to minimize discomfort. Animal suffering will be kept to a minimum by the application of good experimental technique,use of anaesthetics and pain controlling drugs, careful monitoring so to intervene prior to the occurrence of significant suffering

Project 15	**Investigation of normal and aberrant haemopoiesis**	
Key Words (max. 5 words)	Haemopoiesis, cancer, bone marrow failure, graft-versus-host disease	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Haemopoiesis is the process of production of different types of blood cells by haemopoietic stem cells (HSC) and their progeny in an orderly and timely fashion. Depletion of HSC and/or of its progeny can lead to loss of haemopoiesis while uncontrolled growth of HSC and progeny leads to haematological cancers. In many cases the exact mechanisms that lead to failing haemopoiesis or to haematological cancer are not known. In addition, despite recent progress, there remains an overwhelming need for new and more effective means of treatment. The objectives of the proposed project are to: 1. To identify and understand how different genes and proteins lead to failing or malignant haemopoesis 2. To test new drugs and immune cell-based therapies for their ability to treat and/or cure haematological cancers	

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The work we describe here is predicted to provide a number of benefits, both in terms of our understanding of normal and failing haemopoiesis as well as haematological cancers and their treatment. We will provide confirmation of the role of proposed tumour promoting and suppressing genes in haematological cancers, defining the “molecular pathways” that drive these cancers, and thus identify possible clinical markers and novel targets for future therapies. Agents shown to be beneficial in haematological cancer killing, suppressing tumour growth or increasing their chemosensitivity may be taken into clinical trials that should ultimately lead to improved outcome in patients; indeed, we have taken treatments into clinical trial already, based on previous work this study follows on from (I-BET762 clinical trial). In addition, we will be able to develop and optimise experimental cancer immune therapies and obtain safety data required for initiating clinical trials in haematological cancer patients.
What species and approximate numbers of animals do you expect to use over what period of time?	We have confined our experiments to mice, including genetically modified mice. It is estimated that we will use 15,000 mice during the 5 years if the project licence.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Around 80% of mice will experience tumour burden, mild irradiation, multiple imaging sessions, administration of test agents and removal of blood samples. Applying humane endpoints to tumours, careful choice of substances, limitations to blood sampling and allowing the animals sufficient time between imaging sessions will result in a likely severity of moderate. The remaining 20% of mice will undergo, in addition, higher dose of irradiation and risk radiation sickness which will be carefully monitored and humane endpoints applied but nevertheless may risk a severe level of suffering given the other procedures applied.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	All genes, proteins and treatment strategies proposed here will have been thoroughly examined in appropriate laboratory experimental assays using normal- or patient-derived HSC or tumour cells. These assays will identify candidate genes and proteins important for normal, failing or malignant haemopoiesis or candidate treatments which will require validation in animal models. Use of animal models is necessary because: a) Tumour growth cannot be accurately modelled in non-animal models and requires assessment in a fully physiological model system. Further, as the interplay between different cells involved in immune response is complex, in order to study the role of individual immune components in response to tumour, a suitable non-animal model does not exist. b) Functional and safety validation of promising anti-cancer treatments is required using rodent models, prior to their use as novel therapeutics in humans. Mice are to be used here because a mammalian system is required, but higher mammals are not essential.
2. Reduction Explain how you will assure the use of minimum numbers of animals	By involving in the study design a statistician which will ensure we use the minimum possible number of animals in each experiment. By performing pilot experiments to determine the minimal numbers of mice required to give statistically valid results, thereby minimising the number of repeat experiments required By monitoring, in most cases, tumour growth using technology applied to imaging patients' tumours, thus obviating the need to kill mice at defined time-points resulting in a reduction in 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	In order to minimise animal numbers we will use techniques that allow us to monitor tumour growth multiple times in live animals. Mice receiving drugs, immunotherapy or chemicals to induce cancer will also be monitored for adverse signs as described above, and if their condition does

minimise welfare costs (harms) to the animals.	not improve upon treatment they will be sacrificed. Animals will be injected with tumour cells in a small volume of fluid to minimise stress. They will be examined daily for any indications of distress, including changes in weight, deterioration of their appearance, changes in behaviour, breathing difficulties, loss of mobility or lack of normal responses. If such adverse signs are detected the local NACWO and/or NVS will be consulted, and if following appropriate treatment there is no improvement, animals will be sacrificed by approved methods. Mice are the only animal species used in this programme of work. Mice are less sentient than higher mammals but are still relevant to human biology, and in particular normal and aberrant haemopoiesis. Moreover, there is a considerable amount of information in the literature that can be used as baselines and mutated and transgenic strains are available. Pilot experiments and in vivo imaging should ensure that whenever possible mice will be culled before clinical symptoms are evident. Mice will be regularly monitored and killed immediately if they develop signs of distress

Project 16: Biology of Normal and Malignant Blood Cells

Leukaemia; cord blood; fusion gene; cytokine

- Summarise your project (1-2 sentences)

Our projects aim to model key components of the mechanism by which childhood leukaemia arises.

- 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.

In 1988, I proposed that childhood acute lymphoblastic leukaemia (the most common form of paediatric cancer) arises by a '2 hit' process with the first (initiating) mutation occurring in utero and the second some time closer to diagnosis at approximately 2-5 years. Our genetic studies directly on clinical samples have proven this to be correct. It was further suggested that the crucial second or triggering mutations arises as a consequence of an abnormal immunological response to common infections. This idea enjoys substantial support from epidemiological studies. What is required to validate that we do indeed have a plausible causation explanation for childhood leukaemia is a dynamic, in vivo model that can recapitulate the two stages in the disease using human cells. This we are seeking to do with our mouse experiments.

In addition, we are seeking to assess the leukaemogenic potential of the critical subset of cells that drive the process of disease development and evolution – leukaemic stem cells. These can only be assayed in vivo, in immune-deficient mice.

- Outline the general project plan.

We plan to induce normal human newborn cord blood cells to undergo the full leukaemic process by first providing them with a leukaemic gene (*ETV6-RUNX1*) that can initiate a 'pre-leukaemic' clonal expansion and then inducing them to express a natural mutagenic enzyme (called AID) that can solicit the key secondary mutations. We plan to do this in several ways including (i) smuggling in a virus expressing the enzyme; (ii) activating the enzyme naturally by an inflammatory cytokine (TGFβ); or (iii) mimicking an infection, via a synthetic antigen. The human cells are injected directly into the bone (tibia) or intravenously of genetically immune-deficient mice (NSG). We monitor the development of leukaemia by tail vein bleeds and cull mice if and when they show any signs of distress. We then examine the tissues of those mice for full blown human leukaemia. In subsidiary experiments, we transfer actual leukaemic cells from patients directly into NSG mice. By analysing single cell genetics pre- and post-implantation, we can infer the clonal structure or evolutionary phylogeny of the cell population and deduce how

genetically diverse the critical ‘stem cell’ population is.

- 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.

Procedures involve injections of small volumes of cells into mice, either in the tail vein or directly into a leg bone (tibia or femur) under anaesthetic. No adverse effects will arise as a direct result of the injections. Some of the cellular injections will give rise to leukaemia which, if left untreated, would cause significant morbidity and eventual death. However, we serially monitor the blood of mice and check health daily. If leukaemia develops and/or the mice show any signs of distress, they are immediately culled. Some mice may be whole body irradiated prior to cellular injection. At the sub-lethal dose used, we anticipate no harmful effects.

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

If successful, these modelling experiments with mice will endorse our two-hit model for the causation of childhood leukaemia. This would, in turn, encourage a search for a vaccine or other protective prophylactic that would prevent childhood leukaemia.

- 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 use mice strains that have been genetically engineered to be profoundly immune-deficient. This then permits the engraftment of human cells without subsequent immunological rejection. It is the standard model for transplantation of human cancer cells. For both ethical and financial reasons, we plan to use the minimum number of mice necessary. For our two projects combined, we have estimated a total maximum usage of 600 animals. It is likely to be significantly less than this. We use three mice in each replicate group, the minimum acceptable for biological consistency.

- 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.

90% of our research on childhood leukaemia has been conducted directly on clinical samples. However to validate our models for the causation of leukaemia and the role of genetically diverse ‘stem cells’, we require an appropriate, recognised and well-studied in vivo model, i.e. transplantation to NSG mice. There

is no in vitro system that can mimic the process of leukaemia formation. We will continue to focus our major effort on the analysis of the genetic and cellular diversity of leukaemic cells ex-vivo.

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

  As under (4) above, no adverse effects are anticipated (or seen to date) via the initial irradiation of mice or injection of cells. Daily surveillance of the health status of mice and weekly assessment (via tail bleed) of the development of leukaemia ensures culling before the onset of any severe morbidity.

Project 17	**Therapeutic Antibodies for Cancer**	
Key Words (max. 5 words)	Monoclonal Antibody, Immune therapy, Cancer	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objectives of this project licence are to provide data supporting the discovery and development of new treatments for cancer. There have been many advances in the treatment of cancer including the recent focus on therapies boosting the immune system which means that around half the people diagnosed with cancer will survive their diagnosis for 10 years or more. However, many patients do not respond to therapy and tragically die from their disease. There is still a great need for new therapies especially for diseases like non-small cell lung cancer and pancreatic cancer. With the data provided by this project we intend to develop new therapeutics for cancer, bringing the opportunity of long term remission from disease to a larger number of patients than can currently benefit. We aim to generate new treatments, based on a class of drug called monoclonal antibodies. Our antibodies are designed to re-awaken the patient's own immune response to the cancer, much in the same way that an infection by a virus can be	

	successfully resolved and cleared by activating the immune system. During the course of discovering new monoclonal antibodies, we will also be addressing the questions of which patients will respond and whether our treatments can help those who do not respond to the currently available therapies
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The potential benefits of this project will be the progression of new therapies into clinical development and ultimately onto the market bringing benefit to patients. We intend to bring one, perhaps two new therapies for cancer into clinical development over the next 5 years
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 12,500 adult mice over 5 years for 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?	The main expected adverse events will be associated with growing tumours in mice and the use of previously untested treatments. In all cases, the general health and condition of an animal will remain the overriding determinant, in accordance with the National Cancer Research Institute (NCRI) Guidelines for the Welfare and Use of Animals in Cancer Research. We will monitor animals daily and this assessment is done in accordance with the principles set out in the Guidelines for the welfare and use of animals in cancer research: British Journal of Cancer (2010) 102, 1555-1577. Most experiments will involve growing tumour cells subcutaneously so it will easy to monitor tumour size, which is limited to the smallest possible for our studies. The skin can thin over such tumours, but any animals where ulceration of the skin seems likely, or where the tumour interferes with normal function will be humanely killed. When the growth of internal tumours, including metastatic spread is necessary, animals will be closely monitored for distress and tumour imaging may be used.

	For most tumours, the most common adverse effect will be alteration in breathing. Mice demonstrating any evidence of breathing difficulties will be promptly and humanely killed. Advice will be sought from the animal care technician and /or the veterinary surgeon if necessary. The treatments we will use should not cause any adverse effect, but this may occur unexpectedly, especially with new treatments. All novel agents and drug combinations (previously untested) will be assessed using extra monitoring. If adverse events are noted, mice may become hunched/piloerect and may exhibit reduced social interaction and / or mobility. In these situations, an intervention such as the supply of dietary supplements and or the administration of anti-inflammatory by the veterinary may be required to assist recovery, if not, animals may be killed. Adverse events following pre-clinical conditioning or treatments will be assessed by close monitoring of animal well-being and application of the limiting signs documentation. For example If mice demonstrate changes in behaviour, show signs of distress or bodyweight loss of >15% then no further dosing will be given until mice recover. If mice do not recover within 48hrs they will be killed. Finally, in order to protect immunosuppressive mice from adventitious infections, mice will be maintained in Individually Ventilated Cages and manipulations will be carried out under specific conditions to reduce the risk of infection At the end of the experiments, all animals will be killed and tissues taken for further analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The growth of cancer is dependent not only on the tumour cells themselves but also on the interactions with the normal tissue that make up the tumour microenvironment. Indeed, cancers also have dramatic effects across the whole body, affecting how the immune system works. It is not currently possible

	to model all these aspects of tumour biology without the use of animals, as we cannot reproduce the overall complexity of the types of cells involved and how they interact with each other in an in vitro (e.g. using a cell culture) system. We will however test treatments in vitro first, and we will continuously aim to develop novel in vitro assays to test and validate the mechanism of action of our drug candidates. Importantly we will only pick those that have the right characteristics to progress to studies using animals. In this way, for each project, only a few treatments will be tested in animals
2. Reduction Explain how you will assure the use of minimum numbers of animals	Using existing in house and public knowledge we will only limit the in vivo testing to models relevant to the treatment. We will refine and profile each tumour models to unsure that the growth kinetics are not highly variable. We will initially run studies with as few animals as possible. In vivo, We willtest only a few molecules pre-selected on their in vitro characteristics. We will use statistical methodsto ensure that we are using the fewest animals per experiment. We have establish a scientific programme in order to maximise the amount of data obtained from each individual, by conducting several ex vivo experiments to complement the in vivo efficacy data. We will also develop imaging techniques to enable us to monitor tumour growth longitudinally which will enable us to use fewer animals and time points.
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.	Appropriate health monitoring protocols are implemented for mice expected to have an adverse phenotype. When appropriate, suitable analgesia & anaesthetic regimes are used to minimize suffering or the animal is killed by a humane method.. We have chosen Mouse models since they have been successfully used for the development of new cancer therapeutics. The earliest finding that an immune modulating antibody could control cancer growth was made in the mouse and as the immune cells in the mouse and human are sharing high level of similarity, and for that reason there has been a

	large amount of data that has predicted the effects seen in patients. Mice will be housed in state –of –the art conditions with care and welfare provided by an excellent and highly trained team of technicians. We will actively monitor daily the growth of tumours and the impact that has on condition of the animal to ensure that no animal suffers unduly. Importantly we will profile extensively our in vivo models including transcriptomic, genomic and proteomic to ensure we only pick models that are fit for purpose for the mechanism of action of our treatments. We will continue to meet with local and international groups that work in the field on immune-oncology to refine experimental techniques and bring the best advice to bear on our projects so that we can always obtain the best information from the studies we run.

Project 18	**In vivo assessment of biomaterials**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	One in 3 people will develop cancer and new therapies are therefore urgently required. Currently, cancer therapy is utilised in the majority of cancer patients, but could benefit more patients if it were administered in a more targeted fashion thereby sparing healthy tissues, reducing side effects and permitting higher effective doses at the tumour site. One way to achieve this is to develop nano-sized drug delivery systems or by applying focal therapy directly to the tumour. Therefore **aim 1** is to assess functional materials for anticancer drug delivery. However, for solid tumours cancer therapy is typically combined with surgical resection of the tumour. In 20% of patients this results in loss of vital tissue architecture. Therefore replacing lost tissue is attractive to improve patient outcomes (e.g. quality of life, mobility) in the long term. Therefore **aim 2** is to assess materials to replace worn out and diseased tissues. Overall, the ultimate aim of this research is to assist with the development of (i) improved cancer therapy and (ii) better tissue engineering approaches.	

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The data from this project will add to the current knowledge of advanced anticancer drug delivery systems (e.g. nanomedicines, focal therapy) and tissue engineering. This in turn will help us to develop improved drug delivery and tissue engineering systems that will ultimately impact clinical practice.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice will be used in this research. Overall, we anticipate use of 1020 animals in total.
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 associated with these studies are due to surgery, growth of tumours, to the drugs or other substances given to assess their efficacy and to imaging of the animals as disease progresses. Animals will receive post-operative painkillers and will be carefully monitored for signs of illness such as weight-loss or anaemia. The severity is assessed as moderate. At the end of the studies 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	Our research will involve a significant amount of in vitro laboratory work, including tissue culture techniques. This work will ensure that only the most promising interventions are tested in mice. However, studies in mice are still critical for our research because no single tissue culture system can fully mimic cancer or permits a realistic setting to test tissue replacement strategies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments will utilise the lowest numbers of animals consistent with obtaining statistically significant results (discussed with an in house statistician).
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general	Studies are designed so the least severe methods of analysis (e.g. cure of human tumour cells in a mouse) will be used to test promising in vitro schemes. Pilot experiments utilising small numbers will typically be employed in the first instance to establish optimum schedules and to monitor for any unexpected harmful effects. Animals will remain on study for the shortest

measures you will take to minimise welfare costs (harms) to the animals.	time necessary to achieve the scientific data. Painkillers will be used following surgery. Should any unexpected suffering be encountered animals will be humanely killed and subsequent experiments will be amended to avoid further suffering.

Project 19	**Advanced nucleic acid and small molecule therapies for cancers**	
Key Words (max. 5 words)	Nucleic, acid, molecule therapies cancers	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of the research is to develop novel therapies for cancers, especially those affecting children, which can be very difficult to treat. New therapies are urgently needed. We will develop ways of using genes, e.g., switching them off or on, to alter the biological properties of the tumour cells. We aim to find ways of slowing their growth rate or causing them to die off or enabling the immune system to attack the 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)?	This research will teach us how to use nanoparticles to deliver therapies into tumours, enabling very specific uptake of the therapy into the tumour, reducing uptake into other organs. Nanoparticles are made of the therapeutic combined with other molecules that package the medicine into a particle of a size similar to a virus but, being synthetic, with none of the dangers of viruses. This is very important to allow us to develop safer and more effective treatments. This could even allow current cancer	

	drugs to work more effectively. We will also learn a lot about the use of genes to generate new medicines for cancers by increasing or reducing the level of activity of the gene to interfere with the biology of the cells in the cancer, causing them to die or stop growing, Longer term this research could lead to new medicines for cancer which we aim to take into patients.
What species and approximate numbers of animals do you expect to use over what period of time?	We will only use mice for these studies as their biology is well understood and they are widely used. We may use mice that develop cancers of their own or genetically engineered mice that can grow human tumours after we inject a small number of cancer cells under the skin. These types of mice are widely used by other researchers so we can compare our results with those of others. Numbers required will be approximately 120 per annum or 600 over the life of this license.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Mice will experience injections of cells to grow tumours either intravenously or under the skin, They will also receive injections of the treatment being tested which may be intravenous, into the abdominal cavity (intraperitoneal) or into the tumour itself. Some mice will receive repeated injections to see if this is a way to improve the efficacyof the treatment. In some experiments we will anaesthetise the mice then place them in a chamber under a special camera where, for example, we can image the tumour and location of the treatment after injection to make sure it is going into the tumour. These procedures for the mice are of moderate severity, involving different methods of injection for which mice will be anaesthetised and given pain killers such as lidocaine. At the end of the experiments, mice will be killed humanely by a schedule I method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	We will use human cancer cells grown in plastic dishes in the laboratory for many experiments to reduce the need for animals, However the delivery of therapeutics to tumours in patients is very challenging

use non-animal alternatives	and we need to assess tumour specificity in mice first. For example, once injected into the blood the therapy will pass through liver, lungs, kidneys and spleen all of which are very efficient at removing materials from the blood, especially the nanoparticle formulations that we are mainly investigating.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We already have a lot of experience in the kinds of experiments we are planning to perform so we already know how many mice we need to use in a particular experiment to achieve a statistically significant result. For experiments which may be new to us, we will perform small pilot studies and use statistical calculations to determine the number of mice needed. In many experiments involving biodistribution we will perform live imaging using a sensitive camera system available at our institute so that multiple measurements over time can be performed in individual mice, reducing the need for large groups.
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 normal mice where possible as tumour models but where human tumours are to be analysed we will use immunodeficient mice. These mice lack an immune system so that human tumours can be implanted and grown providing a live model of the cancer in which we can test our therapies. Tumours can be implanted under the skin of the mouse on the flanks. This allows easy monitoring and measurement of the tumours by palpation or by eye and in most experiments this is the type of model we will use. In some cases we will want to investigate how cancer spreads in the body to other organs through the blood and if we can treat those tumours. In this case tumour cells will be injected intravenously and the tumours formed will not be visible. Prior to such studies we will perform a pilot study to establish how quickly tumours spread and grow. Mice with injections of metastatic cells (i.e. where the cancer cells may spread through the body) will be particularly carefully observed for signs of ill health, such as breathing difficulties due to metastases in the lung and we will analyse blood samples for signs of anaemia. Mice will be

	anaesthetised to minimise stress and for methods of injection a pain killer such as lidocaine may be used. If there are any unexpected adverse events we will contact the designated vet.

Project 20	**Radiation combinations for cancer treatments**	
Key Words (max. 5 words)	Radiation, rodent, cancer	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall plan of the work is to support the discovery and development of novel cancer therapies to benefit human health in the treatment of cancer. To achieve this we will: 1) Develop and validate IR treatment protocols for the treatment of superficially implanted human or syngeneictumours in mice and rats 2) Determine the tolerability of ionising radiation alone or in combination with novel agents 3) Assess the effects of single or fractionated radiotherapy treatment protocols as used in clinical setting, using animal models 4) Once treatment protocols have been validated the models will be used to evaluate and refine combination treatment regimens with novel targeted therapies to direct clinical development 5) Further test novel cancer agents in combination	

	with radiotherapy to look for additive or synergistic affects using either tumour growth inhibition or pharmacodynamic endpoints.
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)?	Radiotherapy provides significant benefit being used in — over 50% of all cancers. As the patient population treated with radiotherapy is so large, enhancing therapeutic outcome for even a relatively small proportion has the potential to translate into highly significant clinical benefit. To have pre-clinical animal models that will demonstrate and characterise improvements in anti- tumour activity will lead to more relevant potential clinical benefits thus directing clinical development. In this license we will be testing the combination of putative anticancer drugs with ionising radiation with the aim of improving current anti-cancer therapies. With this license we will be able to investigate if new compounds sensitise human tumours to irradiation or if new dosing schedules are better tolerated and/or more efficacious than the current ones. With all this information, new clinical trials can be designed that eventually may change clinical practice.
What species and approximate numbers of animals do you expect to use over what period of time?	Only rats and mice will be used within this project licence. Approximately 2000 mice per year and 200 rats per year will be used over the five year life 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?	Adverse effects related to tumour inoculation may cause brief discomfort or pain.. Adverse effects will be minimised by: • limiting volumes • choice of appropriate needle size • application of good technique by trained licensees The tumour types used are very well tolerated and only one superficial tumour will be used per animal. Tumour size and condition is monitored closely on a daily basis and we will use the least invasive tumour

	site/line that will achieve the scientific aims and will apply the earliest endpoints to meet the scientific requirement of the study. Animals will be culled if the tumour results in significant pain or distress. Clinical signs related to the pharmacological action of the compound may be seen and mild to moderate signs of toxicity are possible. Animals will be humanely killed if this persists. Local irritation at the site of injection may be observed. Animals will be closely observed on the day of dosing. Animals are observed by trained staff, with referral to the Named Animal Care and Welfare Officer, veterinary staff and Project Licence Holder as necessary. All animals will be regularly monitored for weight loss and general condition. Any toxicity associated with radiotherapy will be minimised by careful monitoring and appropriate lead shielding. In most cases there is no toxicity. Weight loss as a result of repeat anaesthesia may occur and this will be minimised by correct dosing, accurate weighing and good maintenance of body temperature during the period of anaesthesia and the recovery phase. Animals that are used where the immune-system is compromised will be housed in sterile conditions. The protocols are classified as moderate severity. Animals will be humanely culled at the end of the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Non-animal alternatives are used in the identification and selection of compounds and generally include measurements of the likely effect of the agent on the target cells or mechanism. Activity in particular cell types however cannot predict the likely in vivo activity given the complexity of issues such as bioavailability, metabolism and elaborate physiological interactions associated with tumour growth. The potential clinical interaction between novel

	therapies and radiation cannot be fully modelled in vitro, given the outcome to radiotherapy is governed by a number of pathophysiological factors in vivo, for example tumour reoxygenation. This drives a need to be able to model interactions with radiotherapy in tumour models in animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To maximise the scientific integrity of data generated and to use the minimum number of animals, in house statistical expertise will be applied to all experimental design and analyses. Where plausible the following statistical guidelines will be used t minimise the number of animals required for each procedure: • meaningful biological change and measurable endpoints will be defined • estimates of biological variability will be used in sample size and power calculations • animals will be allocated in an optimal way based on estimates of biological variability established from accrued historical databases, pilot studies or published data. • regular monitoring and updating of biological databases with regular review of group sizes. • one-sided (rather than two-sided) statistical tests will be used wherever appropriate (e.g. when identifying inhibition rather than change) • statement of intended statistical analyses and justification for use, if any, of transformed data (e.g. tumour growth data may be analysed on the logarithmic scale if the variance of tumour measurements increases with the mean) • statistical power will be set to a minimum of 80% (e.g. at least an 80% chance of declaring the defined 'meaningful biological change' as being statistically significant) • multiple treated groups will be compared against one control to reduce the number of studies

	performed. Group sizes may be weighted to reflect this.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The overall plan of the work is to support the discovery and development of novel cancer therapies to benefit human health in the treatment of cancer. Only rats and mice including immune-deficient strains are used on this licence. Using non-mammalian species of lower neurophysiological sensitivity is not possible since they lack appropriate tissue physiology. Although exact replication of all pharmacokinetic parameters between species is not possible, many features of human PK can be predicted from those observed in small mammalian species unlike effects seen in lower organisms. The most appropriate species and strain of mice and/or rats will be chosen based on previous data that has been used to generate single agent efficacy data. Mice will be used in the majority of studies unless there is a scientifically relevant reason that mice cannot be used, for example, compound metabolism issue with the compound. The choice of strain will be driven by the choice of tumour model. For human tumour lines immune-deficient animals are required to support the growth of the tumour, the least immune-deficient strain required to promote good, reproducible tumour growth will be used. The optimal conditions for tumour growth will already have been developed. For anaesthetic protocols the optimal anaesthetic regime relevant for the species/strain will be developed and used in conjunction with the NVS. Where necessary, pain relief will be used under the guidance of the NVS.

Project 21	**Evaluation of therapeutics for the treatment of head and neck cancer**
Key Words (max. 5 words)	Head and neck, Thyroid, Cancer, Drug discovery
Expected duration of the project (yrs)	5 year(s) 0 month(s)

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

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project will provide a platform for the identification and validation of novel drugs for the management of head and neck and thyroid cancer. We have established extensive and robust laboratory techniques using established head and neck cancer cell lines and ex-vivo primary cultures to screen for and validate targets. Once targets have been identified and confirmed in the laboratory, we need to assess their efficacy and safety in animal models

	before proceeding to clinical trials
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The benefits of these studies are substantial. Treatment outcomes for head and neck and thyroid cancer are poor and are associated with unavoidable toxicities to normal tissue. Side effects experienced by patients include problems with swallowing, distortion of taste and marked residual cosmetic and functional impediments. This project will lead to the discovery of new treatments that would result in higher efficacy or lower toxicity when used alone or in combination with current treatment options. Following on from these studies we will use our extensive experience in setting up and running clinical trials to take any lead compounds into early phase trials
What types and approximate numbers of animals do you expect to use and over what period of time?	We estimate that we will be using no more than 1200 mice over 5 years. Typically, the experimental group size is 4 to 6 mice depending on the type of experiment, to allow for statistical significance. Consultation with a statistician and weekly data discussions within the group will be maintained to ensure the minimum number of animals is always used
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?	The main expected adverse effect will arise from the development of tumours. Mice with tumours may develop enlarged lymphoid tissue and other clinical signs of tumour spread and in the case of sub-cutaneous tumours these may very occasionally ulcerate, in which case the animal will be killed. Substances will be administered in a controlled manner and in concentrations and/or frequency that do not impact dramatically on the general well being of the mice but have an effect on tumour growth. Other potential general adverse effects include minor stress levels due to repetitive handling and injection of substances and weight loss that will not be higher than 20%. No severe adverse side effects are expected from the small surgical procedures included in this license. Well-trained staff will carry out these procedures (such as implantation of a mini pump device for constant controlled administration of novel agents

	under the skin) under aseptic conditions and therefore infections are expected to be minimal. All animals will be closely monitored for adverse effects and will be humanely killed if these are apparent. Multiple analyses will be carried out from a single mouse so we obtain as much data as possible.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-protected animal alternatives	The in vivo work builds on data obtained from extensive optimisation and validation studies carried out *in vitro* . In addition, we will use *in vitro* assays to analyse the functional properties of *ex-vivo* human tissue. However full testing requires the need to find out what happens in the whole, living body, which is far more complex than in cell culture where the cells are removed from their natural environment. There is no appropriate alternative to study the whole animal's metabolic response and the body's handling of drugs and its subsequent metabolites. In vivo studies need to be carried out if the novel anti-cancer agents are to be taken into clinical trials.
2. Reduction Explain how you will ensure the use of minimum numbers of animals	Tumour cell lines will be stored in a frozen state when not being used so that there is no requirement for maintaining a tumour bearing colony. For each experiment, to ensure we only use the necessary number of mice we will calculate the appropriate sample size with the help of a statistician
3. Refinement Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Good laboratory practice, regular review of techniques and constant contact with other fellow researchers will ensure the best model with the least welfare costs to the animal will be employed for this study. All mice undergoing procedures will be closely monitored and the models used are standard and well established in cancer research. Tumour burden will be maintained to the minimum required for a valid scientific outcome. Only the minimum amount of compounds shown to have an effect on tumour growth in preliminary experiments with the lowest welfare cost to the mice will be used for further experiments.

Project 22	**Therapeutic strategies to improve organ quality and function in a pig model.**	
Key Words (max. 5 words)	Transplantation, retrieval, improve organ quality	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of this project is to improve the quality of organs that can be used for transplantation. This is important as many people die whilst waiting on the organ transplant lists. Because of this, more high-risk organs are considered and used for transplantation. We wish to look for ways to assess and improve the quality of these organs, in order to improve outcomes following organ transplantation.	
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 project are that we will be able to assess organs to see whether they can be used for transplantation. We are also hoping that we will find therapeutic interventions that we can use to improve the quality of organs that previously may not have been used. Both of these will increase the number of donor organs available for transplantation and reduce the number of patients that die whilst waiting for an organ.	
What species and approximate numbers of animals do you expect to use over what period of time?	We will be using Pigs and we will be likely to use about 200 pigs in the 5 years.	

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most animals will be sedated and then anaesthetised which will limit any adverse effects to them. However, in the number that we perform recovery procedures, they may experience pain following the surgery that will be treated with painkillers. If the animal suffers any other complication of the surgery, such as infection, bleeding or failure of the transplant, it will be treated appropriately in a timely manner. The animals are only recovered for 5 days, and so they would only experience acute problems rather than any chronic conditions, and as we are regularly assessing them both clinically and biochemically, any suffering should be picked up in a timely manner and dealt with appropriately. If the animal is thought likely to experience continued suffering, then it will be euthanised. At the end of all experiments, the animals will be terminated.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We need to use animals, as they will respond to an intervention in a similar physiological way to humans. Non-animal alternatives would include cell cultures, but these are impractical to test the questions we have about the ways to improve an organ for transplantation. We need to use animals to see the response they have to the therapies we use to see whether they would be likely to work in humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	For all pig studies we are using the minimal number of animals required for statistical significance, following the advice of medical statisticians. Depending on technical aspects this is approximately 6 pigs per experimental group. We are aiming to identify strategies or treatments that show a clear benefit that do not need large numbers in order to demonstrate significance. We will make the maximum use of the results we get, and only carry out bigger surgeries (i.e. transplantation) to validate the findings we get from other studies.
3. Refinement Explain the choice of species and why the animal model(s)	The pig has been chosen for these experiments as it is widely accepted at as the best model for studies that can be related to use in humans. The anatomical and physiological characteristics are similar to those

you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	of humans and the pig is known to be more susceptible to preservation damage than the human. The preservation characteristics of the organs and the perfusion methods that are available are substantially different in smaller animals, and so any experiments done in rodents would not be applicable to humans. To minimise any harm to the animals, analgesia or sedation will be used for all procedures, and they will be closely monitored for signs of distress and suffering. If there are signs of distress or suffering we will intervene to rectify this, or if it isn't possible to treat the cause, the animal will be euthanised.