Animals (Scientific Procedures) Act 1986

Non-technical summaries for projects granted during 2014

Volume 11

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

Project Titles and Keywords

1. **Radiosensitisation of bladder tumours and normal tissues**
    - Bladder cancer, Radiotherapy, Radiosensitisation, Normal tissue effects, Orthotopic bladder model

2. **Using mechanical stimuli to move therapies into tumours and vaccines across the skin**
    - Tumour, vaccine, mechanical, delivery, ultrasound

3. **Virus based Therapies in preclinical cancer models**
    - Adenovirus, Cancer, Oncolytic

4. **In-house service production monoclonal antibodies**
    - Monoclonal antibodies, infections, disease, diagnostics, vaccination

5. **Engineering Immunity to Cancer**
    - Genetic modification, T-cells, Cancer, Therapy

6. **The isolation of novel antibody therapeutics for the treatment of human disease**
    - Antibody, cartilaginous fish, therapeutic drug

7. **Molecular Basis & Theory of Gynaecological Cancer**
    - Ovarian Cancer, gynaecological cancer, immunotherapy, chemotherapy

8. **Developing Selective, Novel Cancer Therapeutics**
    - Cancer, Drug Discovery, Therapeutics, Preclinical

9. **Targeting cancer stem cells and their microenvironment**
    - Cancer stem cells, pancreatic cancer

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

11. **The cause of resistance to treatment in human blood cancers**
    - Blood cancers, resistance, genome-damage

12. **Experimental Cancer Diagnosis & Therapy**
    - Cancer, tumour, diagnosis, therapy, models

13. **Protecting stem cell and tissue function**
    - Stem cells, ageing, drug development, regeneration, mucositis

14. **Nanomedicine Based Drug Delivery**
    - Nanotechnology, Drug Development, Brain Diseases, Biologics

15. **Development of novel agents for cancer therapy**
    - Cancer, novel drugs

16. **Development of personalised anti-cancer strategies**
    - Cancer, Drugs, Translation, Biomarkers, Metastasis

17. **Microenvironment signalling in cancer**
    - Cancer, microenvironment, therapy

18. **Novel multifunctional phage for guided systemic cancer gene therapy**

19. **Evaluation of anti-cancer drugs**
    - Cancer, drugs

20. **Epithelial-Stromal Interaction in Cancer**
    - Cancer, Stroma, Epithelium, Breast, Lung

21. **Biology of relapsed childhood leukaemia**
    - Leukaemia, drug-resistance, relapse, bone marrow microenvironment

22. **Immunisation of Rodents**
    - Immunise antigens antibodies

<table>
<tr><td>PROJECT 1</td><td colspan="3">Radiosensitisation of bladder tumours and normal tissues</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Bladder cancer, Radiotherapy, Radiosensitisation, Normal tissue effects, Orthotopic bladder model</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[1]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">We wish to identify drugs which can be added to radiotherapy treatments for bladder cancer, which would improve the survival in patients following treatment but at the same time not add to the side effects expected when giving radiotherapy alone. To do this, we have to use mouse models to see which agents would be likely to work in humans. We need to find drugs which cause minimal side effects in normal tissues surrounding the bladder and finally, we need to study the agents with radiotherapy in tumours which are located in the bladder, to best represent the patient situation.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be</td><td colspan="3">It is now generally recommended that patients having radiotherapy for their bladder cancer also have a drug or drugs added to make the treatment</td></tr>
</table>

advanced or humans or animals could benefit from the project)?	more effective. However, most bladder cancer patients are elderly and are not fit enough to receive these drugs or do not tolerate the current treatments well. There is therefore an urgent need to find drugs which are suitable for these patients. We can deliver focussed radiotherapy in mice which will allow us to study these agents in a setting which is relevant to the human situation. This will hopefully mean that we can identify and test new drugs to add to radiotherapy, which can be taken forward to clinical trials in patients, and which are likely to improve tumour cure while not adding to the side effect burden.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice and expect to use approximately 2900 over five years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The mice have a <5% risk of dying from a general anaesthetic. Tumours in the bladder may block the tubes to the bladder and cause renal failure, although this is less likely with the technique we shall use. Tumours may spread to the lymph nodes and other organs. Mice could suffer toxic side effects from the agents used in imaging (<1%) or from the test drugs, although mice will not be allowed to suffer and will be killed humanely if there is any sign of this happening. At the end of the experiments, the mice will be killed humanely.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Growing bladder tumour cells in dishes allows us to see if drugs can work in combination with radiotherapy to kill more tumour cells than radiation alone. However, this method does not allow us to test whether the drugs are also having an adverse effect on the normal tissues which would normally surround the tumour area to be treated in humans. Tumours also develop complex surrounding structures to feed and support them in animals and humans, and these cannot be simulated in dishes in a laboratory.

2. Reduction Explain how you will assure the use of minimum numbers of animals	By doing small test experiments with only a few animals, this will let us decide which are the most important larger experiments to perform, which are likely to give us successful results. This will mean that animals are not wasted in experiments which are unlikely to give useful information. We will keep the numbers of animals used in the early test experiments to an absolute minimum, usually 2 or 3 per group. In the larger experiments, we will use a 'factorial design' which means that because of the statistics involved, fewer animals are needed per group to get a meaningful result. Because of new sophisticated methods of imaging the animals, when a tumour is growing inside, rather than having to kill an animal to examine the tumour, animals can be imaged over time, and this means far fewer animals are needed for each experiment.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We use mice as they are the animal species where most is known about how tumours respond to drugs and radiation. We will make sure that the tumour cells that we inject into the animals are the right ones and that they are not infected with germs. In many of our experiments we will inject the tumours cells under the skin in a part of the body that does not cause a nuisance to the animal, and we will not allow the tumours to reach a size that affects the behaviour of the mouse or makes it ill. In some experiments we will create tumours in the bladder wall, but this will be done under anaesthetic by injection under ultrasound imaging, using a very small needle. Again the tumours will not be allowed to grow so large as to cause the animal to suffer. These experiments will be more relevant to the human drug/radiotherapy situation. We will use imaging in some mice to better understand the behaviour of the tumour, but we have limits to the numbers of scans that can be

	performed in each mouse and also complicated scans will be done under general anaesthetic. The drugs will be given in the smallest amount of liquid that is practical and where possible in a liquid that matches the body composition of the animal, to minimise the upset caused. We will not allow mice to suffer, and they will be killed humanely if they show signs of distress.

<table>
<tr><td>PROJECT 2</td><td>Using mechanical stimuli to move therapies into tumours and vaccines across the skin</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Tumour, vaccine, mechanical, delivery, ultrasound</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td></td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[3]</td><td>
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<tr><td>Basic research</td><td>Yes</td><td></td></tr>
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<tr><td>Maintenance of colonies of genetically altered animals[4]</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>To determine if mechanical stimuli can be used to improve the delivery and efficiency of anti-cancer agents and vaccines.</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>Applying a non-invasive, safe, low cost external stimulus, such as ultrasound, magnetism or lithotripsy shock waves, to improve the movement of cancer drugs into tumours and vaccines across the skin will make these treatments more targeted and more effective</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>We expect to use 3000 mice over the next 5-years</td></tr>
</table>

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The systems we are developing are non-invasive and so no surgical procedures are involved. The mice will never be exposed to a levels of severity higher than moderate and in the majority of cases will be exposed to mild levels. Mice will be killed at the cessation of the studies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although we're currently working on it, there are no model systems that can accurately reproduce the complexity of a of the intratumoural environment, especially with respect to the suppression of the immune system which takes place within a tumour.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Careful planning of our experiments and the use of statistical planning will help us minimise the number of mice needed. Animal models and techniques will be used that allow information to be gathered from one mouse over a time-course and so a different mouse will not be needed at each time-point.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the most suitable model in terms of creating tumours and modelling the immune system. Tumour sizes will be prevented from exceeding a defined limit ($1000mm^3$). Pain relief will be provided at cannulation site with the application of EMLA cream Careful training of all staff ensures that the health and welfare of the mice is well regulated and recorded.

<table>
<tr><td>PROJECT 3</td><td colspan="2">Virus based Therapies in preclinical cancer models</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">Adenovirus, Cancer, Oncolytic,</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2">5yrs</td></tr>
<tr><td rowspan="8">Purpose of the project as in ASPA section 5C(3)

(Mark all boxes that apply)</td><td>x</td><td>Basic research</td></tr>
<tr><td>x</td><td>Translational and applied research</td></tr>
<tr><td>x</td><td>Regulatory use and routine production</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="2">Our lab is taking a common virus known as adenovirus and modifying it so it can fight cancer. We seek to tailor the virus so we can deliver it via the bloodstream to tumours, where it will infect only tumour cells and kill them through replication. In this project we seek to:

1. Study how long the virus remains in the bloodstream, and which organs it ends up in when it leaves the bloodstream.
2. Study whether the virus is toxic when given at a range of doses in animals.
3. Establish how much of the virus can reach tumours via the bloodstream across a range of time points.
Overall, these studies will help to inform types of virus for future translational studies in patients.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be</td><td colspan="2">There is a great need to develop new agents to treat cancer, especially as so many tumours rapidly become resistant to existing drugs. Therapies based</td></tr>
</table>

advanced or humans or animals could benefit from the project)?	on viruses (virotherapies) have great potential in this regard, since they possess, through their ability to replicate within cancer cells, the unique advantage that the therapy can be amplified at the point of need. A class of viruses known as adenoviruses, have been studied extensively in the clinic, and are widely regarded as safe, and thus appear to be a promising new class of agent for treating cancers. Despite this, their use is hampered by the fact that the majority of the population have previously been exposed to an adenovirus infection, and thus has immunity against the therapy that rapidly inactivates the virus. Therefore, we are developing new types of viruses to treat cancer that are "tailored" to better infect cancer cells, and are disguised from the host immune system. We have demonstrated that these changes make the virus better at killing cancer cells grown in the laboratory, and now we wish to establish if these developments can be reproduced in whole animals. If successful then these viruses may become frontline medicines in the cancer clinic.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 600 mice over a period of 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The severity limit for both protocols is moderate however we expect the vast majority of mice to experience only mild severity. At the end of the experiments all animals will be killed by a schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Experiments proposed in this project are only those for which there is no *in vitro* alternative. Given that the ultimate aim is to produce therapeutic adenoviruses for use in humans, an intermediate pre-clinical step is required in which to test that the Ads create an effectives response in a whole animals prior to testing in humans.

2. Reduction Explain how you will assure the use of minimum numbers of animals	Experimental designs described in this licence proposal are straightforward and pilot experiments involving small numbers of mice will be used to examine optimal doses and time-courses to be used. The number of animals will also be minimized by performing analyses on the maximum different tissues from each mouse. Where pilot experiments show larger groups are required, power analyses will be used to determine the smallest number of mice required to achieve statistical significance. Where appropriate, a statistician will be consulted during experimental design.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The mouse is very well-defined immunologically and has been used extensively for adenovirus research. It is also the lowest vertebrate group in which these experiments can take place. Together these factors make the mouse the most appropriate choice for these experiments. Animal suffering will be minimal. Variations on these vectors have been used in both human and mouse and found to be well tolerated. The maximum severity allowed for in the licence is moderate; we are confident that this limit is unlikely to be reached even during the acute phase following virus administration. To further reduce animal stress, mice will be kept in social groups wherever possible and are given environmental enrichment such as tubes to hide in and chew, and nesting materials. No procedures of severe severity are proposed.

PROJECT 4	**In-house service production monoclonal antibodies**
Key Words (max. 5 words)	Adenovirus, Cancer, Oncolytic,

- Summarise your project (1-2 sentences)

This application is for an in-house service to produce monoclonal antibodies (mAbs) for researchers in the biological sciences, as part of the Core Biotechnology Services at this University.

- 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.

  Monoclonal antibodies are highly specific reagents binding only to a single target molecule, such as a protein. They are valuable tools in basic research as they can be used to study processes of disease, but also for the diagnostics of bacterial infections. There is a continuous need for these research tools in biology and medicine, and some commercial companies offer a custom service to raise mAbs for the research community. The aim of this service is to work closely with the researchers to identify more accurately the purpose for a new antibody and what it will be used for, than can be provided by a company.

  For example a local project that would benefit from new mAbs is concerned with the study of Neisseria, an organism that is be present in many people without causing disease but under certain conditions can cause meningitis and be fatal. Current vaccines do not cover all variants of Neisseria so the search is on for new vaccines to protect against all variants, and mAbs are essential to progress in this work.

- Outline the general project plan.

  MAbs are produced by immunising a group of mice with an immunogen (usually a protein or protein fragment) and then using the spleen cells of the mouse for fusion with a myeloma (cancer) cell line. This immortal cell line - hybridoma - can be used indefinitely for the production of antibody and stored in liquid nitrogen for later use.

  The aim is to raise mAbs for around 8 projects per year, and to deliver a set of well characterised mAbs for each target within three months from first immunisation. Researchers will have already identified a need for mAbs and by working together from the beginning we will be able to optimise the target immunogen in order to maximise the chances of a good immune response and therefore the quality of the mAb. This will be done by using only a fragment of the protein for immunisation, or by cloning a region predicted to be immunogenic into a carrier protein for “display” of the target, in the form of recombinant protein. Initial immunisation of up to four mice

per target is followed by two boosts and by test bleeds to determine the immune response by measuring the serum titre. The spleens from the best responders will then be used for hybridoma fusion. The best hybridomas will be selected, characterised by appropriate assays to ensure they are fit for purpose, and stable cell lines produced. Antibody will be produced from expanded cultures and handed over to the investigator by testing in their own system. Data sheets will be provided for hybridoma cell lines that have been identified for licencing by commercial companies, with the help of the technology transfer team at the University.

- 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.

  Immunisations with the antigen are done under the skin with a syringe, using a small volume of liquid containing an adjuvant (a chemical that enhances the immune reaction), and this is repeated twice at two weekly intervals. After a further two weeks a test bleed is taken from a surface vein. Finally a terminal bleed by cardiac puncture is done under general anaesthetic, and the mouse killed by cervical dislocation. After this the spleen is removed under aseptic conditions. Adverse reactions such as erythemas, swelling or nodules at the site of injection can occur but are rare with the particular type of adjuvant used

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

  The projects for which mAbs will be raised under this licence are aimed at better understanding the mechanisms of disease or infection, improving diagnostics and at preventing infections by better vaccines. These projects include the diagnostics of bacterial infections by Campylobacter, the organism associated with gastroenteritis from eating under-cooked chicken; Burkholderia, causing chronic and potentially lethal lung infections in tropical countries; the diagnostics and treatment of Pseudomonas and Clostridium infections using host specific phages; and a study of inherited disorders of the immune system causing increased susceptibility to bacterial infection. My current project has been to develop an antibody based test for enteropathogenic *E.coli* (EPEC), an organism that causes persistent diarrhoea in young children mostly in developing countries. The resulting antibodies have been licenced to a company, which has produced a prototype kit for testing children at an early stage of disease, so that lasting damage can be prevented. Another project, to be carried out early, on Neisseria, requires mAbs in the study of vaccine production and optimisation, to increase the coverage of vaccines against meningitis, that are currently not sufficient to protect against all strains of the organism.

- 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.

  Currently mice are the most commonly used animal for raising mAbs as all methodology was developed in this species, and the required fusion partner (cancer cell line also from mice) is available. Mice all react differently to immunisation and more than one animal has to be used to guarantee success. For each antigen I will use up to four animals, and depending on the project, up to three antigens per project. This number is much lower than that used by many commercial companies (10 mice per antigen). Over the duration of the project, estimating an average two antigens each for 8 projects per year, this would involve a total of 320 mice.

- 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.

  There is currently no alternative method for producing mAbs without animals, that can be used routinely, although simple procedures may become available in the future.

  However, by establishing immortal cell-lines that can be used for indefinite production of antibody the repeated sacrifice of animals becomes unnecessary.

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

  The protocol for the animal work in this project is designed to cause the least suffering in a number of ways: using an advanced type of adjuvant for immunisation that causes very few side effects compared with the traditionally used Freund's complete adjuvant wherever possible; all procedures carried out in the absence of anaesthesia are of a mild severity level. As they will be done by experienced technicians they will not cause extended stress to the animal; and the terminal bleed by cardiac puncture will be done under general anaesthetic so the mouse will not feel any pain.

<table>
<tr><td>PROJECT 5</td><td>Engineering Immunity to Cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Genetic modification, T-cells, Cancer, Therapy</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)[6]</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>
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<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[7]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Cancer is currently the second most common cause of death in the UK after heart disease and is predicted to become the most common cause of death in the UK in the next 20 years. Therefore, despite recent advances in the treatment of cancer, there is still an urgent need for the development of better anti-cancer therapies. In the last 10 years it has been demonstrated that a cancer patient’s immune cells can be genetically engineered to recognise and kill their cancer cells but not normal non-cancerous cells. The testing of genetically engineered immune cell therapy in cancer patients has shown that it can lead to total cancer eradication in some patients, but other patients have not responded so well to this treatment. While this approach to treating cancer shows great promise, a number of hurdles need to be overcome</td></tr>
</table>

	for it to become a widely available anti-cancer therapy. Chief amongst these are the need for the further development of genetically engineered immune cell therapy so that: (i) it can be used to treat a greater range and number of cancer patients; (ii) it will be more effective at completely eradicating cancer in treated patients. Therefore, the objectives of this project are: (i) The further development of genetically engineered immune cell therapy to enable it to be used to treat a greater number of cancer patients; (ii) To identify ways of enhancing the anti-cancer efficacy of engineered immune cell therapy so that it is a more effective therapy in treated patients.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The primary benefit of this project will be the further development of genetically engineered immune cell therapy, thereby enabling a greater number of cancer patients to be treated with this promising therapy. Furthermore, this project is expected to lead to the identification of strategies that can be used to enhance the anti-cancer efficacy of genetically engineered immune cell therapy.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will only use mice that have been specifically bred for research purposes. We estimate that we will use up to 960 mice 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?	In addition to normal mice, we will use some genetically altered strains of mice. For example, we will use mice that lack their own immune system as this enables us to introduce a human immune system into these mice The breeding of the genetically altered mice used in this project results in minimal side effects and is classified as being of a mild severity. In this project the experiments we will carry out will involve some of the following procedures: (i) Injecting cancer cells, immune cells and other therapeutic agents into mice using a needle. These procedures will induce some stress due to restraint and transient discomfort from needle insertion (ii) Occasional blood sampling using a needle. This procedure will induce some stress due to restraint and transient discomfort from

	needle insertion. (iii) Limited does of radiation of mice to partially deplete their own immune cells prior to the transfer of cancer-targeted genetically engineered immune cells. High doses of radiation can cause sickness in mice but we will use lower doses that are very well tolerated. In addition to the adverse effects described above, the primary expected adverse effect associated with these experiments is cancer cell growth, which left unchecked will prove fatal. Furthermore, there is the possibility of autoimmune reactions due to genetically engineered immune cells attacking normal non-cancerous cells. Therefore, any mouse showing signs of distress or pain reaching a moderate severity level will be humanely euthanized by an approved method. Finally, at the end of experiments mice will be humanely euthanized by an approved method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Prior to being tested in mice all of the genetically engineered immune cell therapies are tested *in vitro* (i.e. not in animals) to determine if they are able to recognise and kill cancer cells safely and effectively. However, to provide effective anti-cancer therapy genetically engineered immune cells must: (i) survive and expand after transfer into a patient; (ii) circulate through blood and migrate out of blood vessels into the cancer tissue; (iii) migrate through the three dimensional structure of the cancer and kill cancer cells. These complex processes cannot be adequately modelled *in vitro.* Furthermore, well-designed mouse studies can be used to identify unpredictable side-effects that may arise as a result of immune cells killing normal non-cancerous cells. Finally, *in vitro* systems cannot adequately model the complex web of interactions that occur between transferred engineered immune cells and other cell types within the body.
2. Reduction Explain how you will assure the use of minimum numbers	Appropriate statistical analysis will be carried out before experiments to determine the minimum number of mice required for an informative answer.

of animals	Where necessary, we will use pilot studies with small numbers of mice to help determine the minimum number of mice required to reliably obtain an informative answer to the experimental question. By using imaging technologies to visualise both cancer cells and immune cells at different time points in individual mice we can obtain more data from each mouse in an experiment and thereby reduce the total number of mice 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.	Mice have the lowest neurophysiological sensitivity among potentially suitable animal species. Mouse and human immune systems are very similar and the mouse immune system is arguably the best characterized amongst vertebrates. Furthermore, the reagents and tools required for the studies described here have been designed for use in mice. All experiments will be conducted in accordance with the UKCCR guidelines on the welfare of animals in cancer research and we will follow laboratory animal science association (LASA) guidelines. In addition, most of the mouse models and protocols that will be used in this programme of work are well established in our laboratory or those of our collaborators.

<table>
<tr><td>PROJECT 6</td><td colspan="3">The isolation of novel antibody therapeutics for the treatment of human disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Antibody, cartilaginous fish, therapeutic drug</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)[8]</td><td colspan="3"><table>
<tr><td>Basic research</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td><s>No</s></td></tr>
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<tr><td>Maintenance of colonies of genetically altered animals[9]</td><td><s>Yes</s></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Antibodies are naturally occurring proteins in the body that are responsible for binding specifically to foreign molecules and removing them from the body. They are the basis of our immune systems and are necessary to keep us healthy and disease free. Biopharmaceutical companies have identified the usefulness of these proteins to treat disease and there are already examples of effective therapeutic antibodies on the market.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">Mammalian antibodies are relatively big in protein terms and are therefore limited as to which tissues they can access. Just over ten years ago, a novel type of antibody was isolated from cartilaginous fish. These unusual families of antibodies are known as Immunoglobulin Novel Antigen Receptors (IgNARs), which are amongst the smallest known</td></tr>
</table>

	antibodies from the whole of the animal kingdom. The small size and highly stable characteristics of the NARs offer many potential benefits in applications directly relevant to human health.
What species and approximate numbers of animals do you expect to use over what period of time?	Species of cartilaginous fish such as dogfish express this special type of antibody which is only found in cartilaginous fish. The average number that would be used in one year would be 75 with a total over 5 years of 375.
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 protocol is well defined and has been used on this species of animal before so will cause the animal minimal stress and pain. No adverse affects have been noted previously. Smaller species of dogfish will be humanely killed at the end of the procedure. If possible any larger animals will be re-used to minimise numbers, but only after it has been confirmed by a vet with knowledge of the species and individual that the animal is fit and well. Re-using animals avoids capturing and acclimatising to captivity additional individuals and therefore also reduces the overall animal harms.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The antibody that is key to developing new therapeutics is part of the adaptive immune system of these fish. It is therefore necessary to immunize them to initiate the production of these antibodies to the target and then to isolate the domains by taking blood samples.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The immunization method has been used successfully before to develop other treatment molecules and has been optimised so the smallest number of animals can be used to produce the results necessary to isolate the antibody domains.
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	These unique antibodies are only found in cartilaginous fish which includes dogfish. As described above the antibody domains are produced inside these animals in response for a foreign protein being injected – much like our own immune systems. This is essentially creating the

measures you will take to minimise welfare costs (harms) to the animals.	process as vaccinations but against a target found in human disease thereby inducing the animals to raise antibodies against this target. The facilities chosen provide the best care and accommodation for these fish and the team carrying out the procedures have been well trained and are expert in fish husbandry and care.

PROJECT 7	**Molecular Basis & Theory of Gynaecological Cancer**
Key Words (max. 5 words)	Ovarian Cancer, gynaecological cancer, immunotherapy, chemotherapy

- Summarise your project (1-2 sentences)

Every year in the UK around 13,000 women are diagnosed with cancer of the ovaries or uterus, and half of these women will die from their disease within five years. This project will identify new drug targets and will test new therapies for the treatment and cure of these cancers.

Current treatments for gynaecological cancers, whilst initially effective, prove insufficient to cure the patient, and half of all women with these tumours survive less than five years from diagnosis. We therefore require improved drugs or therapies for the treatment of these tumours, or novel drugs that will increase the effectiveness of our current chemotherapies. The studies detailed in this project are designed to firstly identify the genes causing the formation and development of gynaecological tumours, and secondly to test the efficacy of new drugs and therapies designed to target these genes. The successful drugs and therapies identified from this work will then be further developed for clinical trials in cancer patients.

- 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.

The cells in our bodies are controlled by genes that make them grow, or prevent them from growing. If these genes become damaged and do not function properly, our cells can start to grow uncontrollably, forming large lumps called tumours. These lumps break through into the surrounding tissues of our bodies and can spread through our blood to form more tumours spread throughout our body, a disease known as cancer. Gynaecological cancers are those that occur in female reproductive system – such as the ovaries, the uterus (womb), or the cervix. Ovarian and uterine tumours are the most common gynaecological cancers, and represent the fourth and fifth most common cancer type in women. Every year 13,000 women in the UK are diagnosed with one of these conditions. Despite the best efforts of medical staff, at least half of these patients will be killed by their cancer within five years of diagnosis. This is primarily due either to the cancer not responding to chemotherapy drugs at all, or responding initially but then recurring in a chemotherapy-resistant form. We therefore require new and better treatments, or drugs that can prevent the cancer becoming resistant to chemotherapy. The aims of our project are two-fold:

i) to identify the genes that when damaged cause gynaecological cancers;

ii) to test new drugs and therapies for their ability to treat and/or cure gynaecological tumours.

- Outline the general project plan.

Through examining gynaecological tumour material collected from patients during surgery, or from manipulating genes in gynaecological cancer cells grown in the laboratory, it is possible to identify genes that can control the growth of cells, and which have become damaged in the cancer. However, to prove that these candidate cancer genes are truly involved in causing a tumour to grow in the body it is necessary to examine the gene's function in a living organism. If cancer cells are injected into a mouse with a defective immune system the cells are able to grow into a tumour, closely resembling the process seen in humans. Once a candidate cancer gene has been identified in the laboratory it is possible to take cancer cells in which that gene is damaged and genetically modify those cells to "repair" that damage. If these "repaired" cancer cells are injected into mice, we would expect that they would show a reduced ability to grow into a tumour, or even be unable to form a tumour at all, as compared to the "damaged" cancer cells. If this is seen it confirms that the candidate cancer gene is truly important for the growth of gynaecological tumours. These same mouse models carrying tumours can then be used to test novel drugs or therapies designed to target those damaged cancer genes. Successful new treatments should result in decreased tumour growth in the animal models, or increased sensitivity of the tumours to simultaneous chemotherapy treatment. Proving the efficacy of a new therapy in treating tumours in a living organism is essential before the drugs can be trialled in human patients.

These studies are designed to identify genes involved in the growth of tumours, and then subsequently test new drugs that target these cancer genes. The outcome of this work is the identification of new treatments which can be taken into clinical trials to improve the survival of patients with gynaecological cancers.

Tumour growth is a process of living organisms and involves the interaction of tumour cells with the surrounding tissues, blood vessels and immune cells. As such it cannot be accurately modelled in the laboratory. Initial studies from work on tissue samples or cells grown in the laboratory will suggest likely candidate genes involved in cancer development. However, many cancer genes function by regulating the interaction of the tumour cells with the surrounding tissues or with immune cells, or promote the growth of blood vessels into the tumour mass, and therefore confirmation that these truly contribute to tumour growth requires examining them in living cells in a living organism.

Similarly, initial work on testing novel anti-cancer drugs can be done in the laboratory, but subsequently it is necessary to use animal models. This therefore demonstrates that the drugs can successfully reach the tumour and interfere with its growth in its natural setting, as occurs in patients.

The identification of candidate cancer genes that are involved in the development and growth of gynaecological tumours will be initially identified from studies on cancer cells grown in the laboratory, or on tissue samples collected from patients undergoing surgery. Manipulating those cancer genes in cells in the laboratory will then provide us with some information about their function in both normal and cancer cells. Once these cell culture studies have prioritised the most promising candidate cancer genes we will then turn to animal models, injecting mice with cancer cells containing damaged or repaired versions of these candidate genes and comparing their ability to form tumours.

Confirmation that these candidate genes are truly important in the development of gynaecological tumours is the first step in designing improved drugs and therapies to treat cancer patients. To do this, we will first return to cell culture experiments in the laboratory. Taking known drugs or newly developed drugs that target the cancer genes we identified through the above experiments. We will add these drugs to the cultured cells and determine that they are able to kill the cancer cells, or increase the sensitivity of the cells to killing by standard chemotherapy drugs. Once we have proof of principle that these drugs would be effective at treating cancer cells we will again turn to animal models. Using mice injected with cancer cells so that they grow tumours, we will treat the mice with our drugs of interest and look to see whether they can truly result in decreased growth or even cure of the tumours. Our specific interests, as described in this project, are in demonstrating the benefit of drugs that interfere with the interaction of tumour cells with the surrounding tissues, or drugs that overcome the resistance to standard chemotherapy thus increasing its effectiveness. A third type of therapy that we will also use in this project is immunotherapy, where we will manipulate immune cells in the laboratory and then inject them into the tumour-bearing mice. The intention is that by manipulating the immune cells we can increase their ability to locate and destroy cancer cells, thus treating and possibly curing the mice. The drugs or immunotherapies that show efficacy in treating cancer in these animal models will then be developed further for clinical trials in humans to determine that they are safe and effective anti-cancer agents in humans as well. We expect this work to ultimately improve the treatment and outcome of gynaecological cancer patients.

- 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.

Mice will first be injected with cancer cells, either subcutaneously or into the abdominal cavity. They may subsequently be injected with either drugs of interest (with or without standard chemotherapy drugs), or with modified immune cells, to determine whether these treatments decrease the tumour growth. Subcutaneous tumour growth in the test and control groups will be measured visually using callipers. Abdominal tumour growth may be measured weekly using bioluminescence imaging by injecting them with a chemical called luciferin and then

using a special bioluminescent imaging camera to photograph and measure the tumours growing in their abdomen. In addition, monitoring of the tumour may be combined with tracking of immune cells that have been injected into the animal and are used here as treatment. The immune cells will be tracked using bioluminescence imaging with two different chemicals, or in a few animals using CT/PET scan and a different chemical. The imaging is done under general anaesthetic. This will provide us information about response of the animals to this treatment as well as availability and effect of immune cells directly in the tumour tissue. This is important information if we are to plan this treatment in humans as it will provide us information as to how long these cells remain active in the tumour tissue also how often the treatment would need to be repeated if at all.

Animals may show signs of distress, including changes in weight, deterioration of their appearance, changes in behaviour, breathing difficulties, loss of mobility or lack of normal responses.

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

The work we describe here is predicted to provide a number of benefits, both in terms of our understanding of tumour biology and in how we treat gynaecological cancer. We will provide confirmation of the role of proposed tumour promoting and suppressing genes in gynaecological tumours, defining the "molecular pathways" that drive these cancers, and thus identifying possible clinical markers and novel targets for future therapies. Agents shown to be beneficial in tumour killing, suppressing tumour growth or increasing tumour chemosensitivity of gynaecological cancers may be taken into clinical trials that should ultimately lead to improved outcome in patients and indeed we have taken treatments into clinical trial already, based on previous work this study follows on from.

- 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 would estimate using up to 2000 mice for these tumour growth experiments each year. The minimum number of animals required will be used to detect a significant difference between treatment groups. We have ensured that our protocols are designed such that maximum information is gained from each experiment reducing the need for repeats. Tumour in the animals is tracked but regular measurement or imaging at different time points in order to maximise information obtained about a particular treatment and reduce number of animals that is required. For all experiments, a statistician has been consulted to determine the minimum number of animals required to obtain a statistically proven answer to whether the gene or drug of interest is able to decrease tumour growth. For experiments where tumour cells are injected into the mice, 9 animals are required in each of the test and control

groups.

Mice are to be used because a mammalian system with a female reproductive system closely resembling that of humans is required. Their cancer biology and genetics is also well characterised and similar to those of humans. For those experiments dependent on growing human tumour cells in the mice, the use of immunodeficient animals is required. The best characterised of these is the Nude or SCID mouse. The use of the Nude or SCID mouse as a model of gynaecological tumour growth is well established and robust, and the tumour growth is efficient, thereby minimising the numbers of animals that need to be used.

Likewise, a fertilised chicken egg model (Chicken CAM model) can be used, in combination with some of the mouse experiments, to confirm results in an in vivo system before proceeding in the mouse. Approximately 1000 eggs will be used a year and this will allow a more efficient and productive use of mouse experiments. The chicken egg model is widely used to study blood vessel formation, metastasis and in anti-cancer drug screens.

- 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.

All genes and therapeutic strategies used in this work will have been thoroughly examined in non-animal models wherever possible and determined to be important candidate cancer controlling genes in gynaecological tumours or candidate targets for therapeutic inhibition. However, tumour growth cannot be accurately modelled non-animal models and requires assessment in a fully physiological model system. All drugs used in this work will have been thoroughly examined in non-animal models and determined to successfully target candidate proteins or immune cells. Functional validation of these drugs in the treatment of tumours is required using rodent models, prior to their use as novel therapeutics in humans. 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. In addition the limited information obtained from non-animal work on the function of immune cells does not provide safety data. Functional and safety validation of these strategies in the treatment of tumours 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. We have discussed our study designs with a statistician to ensure we use the minimum possible number of rodents in each experiment.

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

Animals will be injected with tumour cells in a small volume of fluid 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 a Schedule 1 method. 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 not improve upon treatment they will be sacrificed.

<table>
<tr><td>PROJECT 8</td><td>Developing Selective, Novel Cancer Therapeutics</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cancer, Drug Discovery, Therapeutics, Preclinical</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)[10]</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[11]</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>Cancer remains a leading cause of mortality worldwide with 160,000 deaths in the UK each year. The major killers are: lung, bowel, breast and prostate cancer. These often have limited treatment options and mortality is frequently linked to metastatic spread to other vital organs. Therefore, there is an urgent need for new and better therapies.

Our aim is to discover and develop more effective, novel, and better tolerated therapies, primarily by targeting the genetic make-up of the patient’s tumour, causing less damage to non-tumour tissue and reducing side-effects. This will help address the current need for new treatments and improve the management of cancer as a life-threatening</td></tr>
</table>

	disease. This programme of work will be carried out to provide 3-4 clinical candidate drugs within 5 years. Each will be tolerated at a level related to activity against the intended drug target that will result in anti-tumour activity, which can only effectively be measured using animal models.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We are leaders in a new field of cancer biology. We have been successful in generating clinical candidate drugs in a related area and will apply that knowledge and expertise to this field. This work is original and will generate novel anti-cancer drugs that can target specific mutations in a patient's tumour allowing the treatment to become more personalised and effective. Furthermore, these new drugs will offer fewer side-effects than conventional treatments creating better quality-of-life for patients. Our new treatments may also be given in combination with existing treatments such as chemotherapy, radiotherapy or novel treatments. In summary, we will: (1) generate new cancer therapies so that patients with cancer can be treated more effectively and safely; (2) advance scientific understanding of cancer biology.
What species and approximate numbers of animals do you expect to use over what period of time?	We will only use mice or rats bred for laboratory research. In 5 years we expect to use approximately 20,000 mice and 4,000 rats.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Based on experience and veterinary advice, animals might experience mild pain from injections but this will be short-lived. Some animals will be used in surgical procedures but these will be conducted under anaesthesia and pain relief will be provided. The majority of animals are expected to make a full recovery. A large number of animals will develop tumours; a fraction of these tumours may show signs of ulceration or inflammation. These will be closely monitored on a daily basis. Animals will be humanely killed before the tumour burden causes lasting pain or harm. If animals

	show signs that depart from normal behaviour or feeding/drinking patterns then they will be assessed and, if necessary, killed humanely. The majority of animals will not exceed a moderate severity limit; on rare occasions (~2%) it is possible that some animals will experience unexpected severe side-effects. If these cannot be treated or do not improve, then we will ensure the animal doesn't suffer and is killed humanely. To minimise the number of animals affected, we will use very small numbers in these experiments and assess them regularly. Animals will be given pain relief where appropriate. Animals will not be allowed to suffer lasting pain or harm. At the end of every experiment, animals will be killed by an approved humane method. Unique genetically-engineered mice may be provided to other researchers to prevent duplicating the same mice.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our drug discovery programme uses many non-animal techniques such as cell-free biochemical assays, cell assays and computer modelling to address important early-stage criteria such as drug potency, selectivity and potential side-effects; metabolic activity is often initially assessed using liver preparations (artificially, outside the animal). While these reduce the numbers of animals used they do have limitations and cannot (currently) fully replace animal models. Additional biological complexity is created in terms of the disease itself. For example, at least one of our targets is highly over-expressed as a cancer-causing gene and so complex biological models of tumour growth are needed and these require living systems. Since humans share the vast majority of biological pathways and DNA sequences with other mammals (mice and rats being the lowest appropriate species) we can use them as surrogates to show that active drug levels can be achieved at safe doses before clinical studies are conducted in

	patients.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our non-animal alternatives help in reducing our animal usage. Only new drugs that make it through strict criteria in non-animal experiments will be progressed to testing in animals. Before we perform a series of experiments in animals, we will conduct smaller pilot studies. We will use statistical methods to analyse experimental data to ensure that we use the least number of animals possible in order to achieve our scientific objectives. Where there is a need to use genetically modified mice, we will obtain these from other researchers thereby reducing animal numbers.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice and rats are extremely well characterised biological model systems. While they do not fully reflect all the aspects of cancer in humans, they remain valid, useful and the most refined of research models for cancer researchers. Lowering the risk of administering an unsafe drug or unsafe levels of a drug to patients is the main reason for testing drugs in animal models but these also serve to predict if the drug will work. For therapeutic studies we will develop tumour models that recapitulate the patient's tumour e.g. tumours transplanted into immune-deficient host mice (to prevent tissue rejection) or tumours developing in mice whose DNA has been altered to drive human-like tumour formation. We will strive to focus on animal welfare in our studies and will make refinements wherever possible, including the latest innovations in environmental enrichment, refinements in dosing techniques and applying best practice for surgical techniques, pain relief and anaesthesia. Consideration of the 3Rs is an integral part of planning experiments and these will be implemented throughout our project.

PROJECT 9	**Targeting cancer stem cells and their microenvironment**	
Key Words (max. 5 words)	Cancer stem cells, pancreatic cancer	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals[12]
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The purpose of our research is to improve the still dismal outcome of patients with pancreatic cancer. Despite increasing research efforts, pancreatic cancer remains the most lethal human cancer and is the 4th most frequent cause of cancer-related death due to lack of efficient treatment strategies. At advanced stage with tumour dissemination to liver and/or lung, at which 90% of the patients present at the time of diagnosis, virtually all patients succumb from disease within 12 months. During this time, many patients also suffer from rapidly declining performance as well as dramatic weight loss. Therefore, pancreatic cancer represents a major socioeconomic and humanitarian challenge. In order to address this major unmet medical need, we need to discover new and more effective ways for treating patients with pancreatic cancer. This will also require carrying out research on live animals. Over the past 5 years we have strongly contributed to expanding the basic understanding of pancreatic cancer stem cell biology and function. These cells	

	represent a small subset of cancer cells, which are characterized by extraordinary aggressiveness and high resistance to current standard treatment. This now enables us to carry out the necessary experiments to identify new therapeutic targets against these very aggressive cancer stem cells. For this purpose, we will use genetic tools to identify novel genes, which could be necessary for the cancer stem cells to function properly. Subsequently these identified candidate genes need to be confirmed, which will first bë done using innovative ceft culture models. However, eventually animal studies will be essential to verify the important role of these newly identified genes in living organisms. These animal experiments are also required by regulatory authorities before any trials of new drugs can be tested in humans. But it's important to note that our novel approach bears strong potential to eventually improve the prognosis of patients with pancreatic cancer. This claim is based on our previous work demonstrating that only if we are able to also eliminate cancer stem cells we may eventually be able to achieve cure from the disease. Therefore, our data clearly demonstrate that cancer stem cells signify a crucial component for any novel treatment approach. Of course animal studies will only be performed after every feasible test has been conducted on cancer cells in the laboratory and where no alternative exists. Only the most promising therapeutic targets will be validated in animals using mouse models that are most relevant to human disease. It is important to note that a single drug is unlikely to cure pancreatic cancer, but our studies are designed in a way that will enable us to develop novel combination therapies in pancreatic cancer. The ultimate goal of our work is to translate these findings into the clinic to eventually improve the outcome of this currently lethal disease.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Our proposed study will embark on developing new combined approaches for improved cancer therapy. Our findings will not only contribute to enhancing our understanding of cancers stem cell biology, but also to improving treatment response in patients with pancreatic cancer. Specifically, for the most promising candidate genes identified in our animal studies we would perform high-throughput searches for new drugs using large libraries of existing and new

	compounds. Identified drugs from these screens could be further improved and then validated as novel compounds. Eventually, we aim to use these novel drugs in combination with biomarkers predicting treatment response in patients are most likely to respond (personalised medicine).
What species and approximate numbers of animals do you expect to use over what period of time?	Each year we will use approximately 6000 mice in our breeding procedures and 2500 mice in experimental procedures. We use the minimum number of animals required for experiments to be statistically sound.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	In this project mice will generally undergo procedures inducing cancer-related inflammation so it is anticipated that the expected adverse effects will be that mice will develop tumours and/or a degree of inflammation. The tumours may be spontaneously occurring in animals, which have been genetically altered/selected to produce pancreatic cancer or the tumour cells will be injected into the mice. We will keep suffering to a minimum with the use of relevant welfare end points. Staff working with the animals will be familiar with the clinical signs that might occur and with these endpoints. When designing experimental protocols, the animals' welfare and the minimisation of suffering are both at the forefront of design. We include wherever possible, the use of anaesthetics and analgesics and non-invasive techniques. No animal is expected to experience more than moderate severity.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Pancreatic cancer is characterized by a massive amount of surrounding connective tissue. This connective tissue does not only represent a structural framework, but is also composed of active ingredients including immune system, vascular cells and activated fibroblasts, all of which actively involved in cancer grow and spread. To study the tumour including its surrounding environment it is necessary to use mouse models as this complexity cannot be reproduced in currently available tissue culture systems. Alternative three-dimensional cell culture models of the pancreatic cancer that uses malignant cells and

	stromal cells is a useful alternative for some of our studies and will be applied whenever possible. However, we cannot include immune cells, the cultures only last a few days and it is very difficult to have all the cells from the same individual. Therefore, it's utility for pre-clinical studies is very limited and cannot replace animal studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have extensive experience in calculating numbers of animals needed to obtain meaningful results between test and control groups, have consulted statisticians on existing experimental designs and will consult again on any new designs. In general, we design our studies to allow for the detection of differences of 25% as the minimum requirement for a new treatment strategy to be promoted in our lab to the next stage of development. We use non-invasive imaging technologies which mean we can reduce the number of animals required for each experiment as this removes the need to kill animals at different time points to observe tumour development and treatment response.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We will use mice in this project. Wherever possible we will use genetically altered mouse models of pancreatic cancer, this enables us to use mice without the necessity of using invasive procedures to induce tumour growth and/or inflammation and will also give us spontaneous models of the disease including the an intact immune system. All of our mice are kept in modern housing conditions and are inspected at least once daily. We have also successfully developed minimal invasive techniques that enable us to inject patient-derived tumour cells directly into the pancreas, therefore replicating human disease more accurately, but reducing the stress to the animals to the minimum that currently can be achieved. These procedures are always performed under general anaesthesia and analgesia is given as routine. We will continue to develop these techniques further and we also have specifically labelled cells, which allows us to study the growth and spread of the disease by non-invasive imaging techniques. We will continue to use imaging methods wherever possible and look at implementing further technologies that will allow us to minimise animal suffering.

<table>
<tr><td>PROJECT 10</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)[13]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[14]</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>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</td><td>The imaging methods and techniques will be used to assess novel drug safety and efficacy in drug</td></tr>
</table>

project (how science could be advanced or humans or animals could benefit from the project)?	development, including new cancer drugs. Data 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 11</td><td colspan="3">The cause of resistance to treatment in human blood cancers</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Blood cancers, resistance, genome-damage,</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)[15]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[16]</td><td></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">1. Advance our understanding of how blood cancers progress.

2. Devise new treatments that are less toxic and more specific for these tumours.

3. Do this by testing whether resistance to treatment is a consequence of the cell losing a particular type of cellular function– one that would normally allow cell death.

Our cells, normally, have active cellular functions-pathways- that respond to damage to our genetic material (our DNA). This pathway may make the cell stop dividing, repair the damage and then, later, carry on dividing. Or it may take a more drastic action, which would be to kill the cell if the damage</td></tr>
</table>

	was too great. If these pathways were not functioning there would be no rescue of damaged cells and no write off of cells that were too damaged. This causes accumulation of genomic errors and makes a tumour cell resistant to treatment and more likely to survive. 4. Find treatments that will work in this situation
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	An improved knowledge of how blood cancers such as chronic leukaemia develop and become resistant to treatment. An improved knowledge of how we might treat blood cancers and in particular chronic leukaemia in patients In the longer term the potential of an improved survival time for patients with particular forms of blood cancers
What species and approximate numbers of animals do you expect to use over what period of time?	Mice Numbers to be used over 5 years = 3,200
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?	With the intravenous administration of tumour cells, the adverse effect will be stress due to restraint and transient discomfort from needle insertion. For subcutaneous and intra splenic administration anaesthesia will be used. With the growth of the tumour, lethargy, enlarged spleen, diarrhoea, weight loss, hunched back and starey coat may be apparent. Subcutaneous injection my result in skin ulceration. There may be infection at the site of the wound. Animals will be sacrificed at these stages. With the administration of anti cancer agents the *expected side effects will include, l*ethargy, anaemia, bruising, loss of appetite, diarrhoea, drop in body temperature or reduced peer interaction in all animals. With irradiation, damage will occur to the blood system and bowel in a small proportion of animals causing diarrhoea, loss of appetite,

	hunched back, starey coat and loss of response to the environment. Animals in this condition will be sacrificed. The likely level of severity will be moderate. At the end animals will be humanely killed by Schedule 1 Method. At this point restraint will cause further stress to the mice.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Chronic leukaemia cells are very difficult to grow in isolation in tissue culture. We have not discovered the key factors that will enable this to occur in tissue culture. These tumour cells require factors from the environment, (stroma), of the tumour (the normal marrow or a lymph node) to grow and survive. The treatments that we propose must be shown to work in animals before they can be tested on people. We have undertaken something similar, previously, which was to test a drug called a PARP inhibitor in animals. We are currently using this drug in a trial to treat people with chronic leukaemia. We will only undertake animal work at all, once we have evidence from other cell culture systems in vitro that a desired outcome, in terms of killing cells can be demonstrated for any treatment.
2. Reduction Explain how you will assure the use of minimum numbers of animals	By designing the experiments correctly, and taking statistical advice, we can use the minimum numbers of animals required to carry out the work and obtain a statistically significant result from the number of animals used. The number of animals (9-12) per arm for each cohort is calculated on the basis of statistical power (80—90%) to identify discrete changes of 15% in tumour growth. In cases where a higher impact of new treatments is expected (based on *in vitro* data), the number of animals will be reduced accordingly. We will also maximise the results that we can obtain from each animal. We will report our results according to NC3R's

	'Animal Research: Reporting *In Vivo* Expts.' In order to inform others of this work and hopefully avoid duplication of animal work.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We will use immunodeficient mice, as we are doing currently. These system models are widely accepted for establishment of grafts of human leukaemias and lymphomas and so far they have generated a wealth of data related to the progression and therapeutic response of haematopoietic malignancies. We will minimise harm to animals by only carrying our approved procedures in an approved manner and housing them in a Unit staffed by professionals who will not allow animals to be distressed.

PROJECT 12	**Experimental Cancer Diagnosis & Therapy**	
Key Words (max. 5 words)	Cancer, tumour, diagnosis, therapy, models	
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[17]
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This program aims to develop improved anti-cancer therapies and diagnostics as well as models which are predictive of clinical tumours.	
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 remains one of the leading causes of death and the lifetime chance of contracting cancer in the developed world is as high as 1in 2 or 3. Nevertheless novel drugs and improved diagnostics have been demonstrated to have significant clinical impact resulting in earlier discovery and moreefficacious treatments. However, despite the success there is clearly a medical need to develop improved therapeutic and diagnostic strategies. We have developed novel therapies and diagnostics based on our understanding of the molecular basis and biology of the disease. These therapies have already shown significant promise in bringing improved selective cancer therapies to patients. However, new potential drug molecules and systems for their delivery to the tumour need to be tested in animals to ensure that they are efficacious before	

	they undergo further development. This happens after cell based assays have been used to weed out those compounds which are less promising. However, cancer cells growing in isolation in the laboratory can only give information about some aspects of how drugs are going to behave in actual tumours or inside a patient. Scientific and technological advances mean promising compounds and devices are being developed but to accelerate the transition of these into the clinic it is important to select only the most promising for further clinical development. New molecules and systems for the delivery of therapies are first tested and developed in cell based assays. However, cancer cells growing in isolation in the laboratory can only give information about some aspects of how drugs are going to behave in actual tumours or inside the body and eventually it becomes necessary to test the most promising systems in animals to ensure that they are efficacious before they undergo further development. Although animal models of human disease have been instrumental in facilitating the medical advances we benefit from today they are imperfect representations of the clinical situation in humans: they accurately replicate some aspects of human cancers but because of underlying differences in the biology are less predictive of other elements. Another part of our research is therefore to make sure that the animal models are developed and characterised which most closely resemble the key aspects of human tumours relevant to the preclinical evaluation of a particular candidate therapy or diagnostic. The choice of the mouse as the model species in cancer research has the benefit that many models already exist which already have been well characterised. The development of better animal models and biological markers directly impacts on our ability to make pre-clinical models more predictive and thus has potential benefits to patients, drug developers and cancer scientists, and the pharmaceutical industry; however more relevant animal models also have an animal welfare and ethical dimension as they embrace the implementation of the 3Rs. Ultimately a better understanding of the those aspects of a model which are predictive of the clinical outcome will also lead to a significant reduction of animals entering into pre-clinical experiments and safety and safety

	pharmacology studies.
What species and approximate numbers of animals do you expect to use over what period of time?	3000 mice over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The severity levels of the procedures are mild to moderate. Typically, the procedures have a limited duration and the animals are monitored regularly for any signs of adverse effects. Therapeutic and diagnostic interventions are of a similar severity to what a human patient would experience in a hospital. Animals are humanely 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 severity levels of the procedures are mild to moderate. Typically, the procedures have a limited duration and the animals are monitored regularly for any signs of adverse effects. Therapeutic and diagnostic interventions are of a similar severity to what a human patient would experience in a hospital. Animals are humanely killed at the end of the procedure. The behaviour of cancer cells in a tumour differs from that of individual cells. The interactions of drugs with the body cannot be tested by other means. In vitro assays do however serve as useful tools in the pre-selection of active compounds and allow the determination of safe starting concentrations for dose ranging studies. To the extent that they are applicable these types of studies routinely precede any work with animals. However, some drug properties and activities can only be understood in the context of the body. This includes the way the body deals with the drug (its distribution and elimination for example) but also the testing of systems that aim to maximise the amount of the drug reaching the site of the disease. The most promising systems therefore need to also be evaluated in vivo before being considered for uses in patients.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will utilise pilot experiments with small animal numbers to optimise procedures. Main studies are carried out based on understanding gained from initial experiments in cells. Application of statistical methods will help to reduce number of animals required to answer a specific question.

3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the most established model animals for tumour work. The type of tumour models will be optimised so that the most appropriate model is used to answer a scientific question. Procedures will be carried out by trained personnel at a level of care that would be expected in a veterinary surgery. The use of a stereotactic frame to stabilise animals during intracranial tumour grafting may cause pain that should be controlled by anaesthesia; on balance the stabilisation and improved reproducibility of the procedure are thought to represent a important refinement of the procedure.

<table>
<tr><td>PROJECT 13</td><td colspan="3">Protecting stem cell and tissue function</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Stem cells, ageing, drug development, regeneration, mucositis</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[18]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[19]</td><td></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">1.To understand how stem cells lose function, particularly with age or when exposed to damaging agents and to identify drugs which delay or prevent stem cell loss of regenerative capacity

2. To determine whether this approach can prevent radiation induced mucositis</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">We will identify new drugs capable to maintain stem cell regenerative capacity when they undergo damage. An example is when a cancer patient undergoes radiotherapy and the blood and intestine are severely damaged. We hope to find drugs which prevent side effects of radiotherapy while still retaining their effects on cancer cells.</td></tr>
<tr><td>What species and</td><td colspan="3">We will use mice, 100/year over 5 years</td></tr>
</table>

approximate numbers of animals do you expect to use over what period of time?	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The animals may be sick (similarly to what cancer patients undergo radiotherapy) with diarrhoea, weight loss and problems of mobility. If the effects are too severe we will sacrifice the animals. At the end of the experiment the animals will be killed and their tissues examined and analysed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is not possible to study the full array of regeneration outside a living organism. There are no in vitro assays that reproduce tissues in all their complexity. Moreover, no in vitro system can predict how the drug will circulate in the body and distribute to the organs of interest and for how long it will be effective
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will plan the experiment carefully and predict the number of animals using statistical tests. We will take professional advice from experimental design experts.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the most commonly used animals to study regeneration and for drug testing. This means we have lots of information about them, which allows us to design better experiments. We will monitor the adverse effects very closely, give pain killers to the animals if required or sacrifice the animal if the adverse effects are too serious

PROJECT 14	**Nanomedicine Based Drug Delivery**	
Key Words (max. 5 words)	Nanotechnology, Drug Development, Brain Diseases, Biologics	
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[20]
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To test the ability of nanomedicines (nanoparticulate medicines) to enable the drug to reach its therapeutic target in the body at such a concentration as to elicit an optimum pharmacological effect and minimise drug side effects. This will allow drugs to be developed to treat brain diseases for example. Currently many drug compounds cannot be used to treat brain diseases as they do not access the brain when administered.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	If nanomedicines are shown to enable drugs to achieve their full pharmacological effects, this could lead to drugs being developed for diseases such as Alzheimer's Disease and brain cancers. Currently these diseases are incurable and one of the reasons for this is that most drugs do not reach the brain when administered by injection or by mouth and all biological type drugs such as antibodies do not reach the brain no matter how they are administered.	

What species and approximate numbers of animals do you expect to use over what period of time?	Over the five year licence period, I expect to use no more than 6000 mice, 4000 rats and 700 rabbits.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Animals will be subject to a number of procedures (induction of acute and chronic pain, polycystic kidney disease and inflammation of the eye) that will be kept within a moderate severity limit by use of aseptic surgical technique, anaesthesia and analgesia where appropriate, careful monitoring, use of clinical score systems and application of appropriate humane endpoints. Careful study design will reduce the risk of side effects of new drug formulations (escalated dosing). Other measures (blood sampling, administration of substances, behavioural cognition tests and standard nociceptive testing) are expected to have mild severity limited by applying appropriate limits. Animals will be humanely killed at the end of each study using a regulated method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Testing where medicines go in the body when administered or how they exert their pharmacological effect, as a result of a change in their destination in the body, is only possible in living animals. As these medicines ultimately will be used to treat human disease, the lowest form of mammals that can be used will be employed, i.e. the rodent and rabbits.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will make sure that we have the right numbers of animals to be able to obtain high quality data each time we do our experiments so that we do not have to unnecessarily repeat studies. We will combine some observations to a single cohort of animals so that we do not have to use additional cohort of animals for each single observations. We will include go/no go decision points at various stages of the project. These decision points will be informed by laboratory data. This will prevent the use of animals in otherwise redundant experiments.
3. Refinement Explain the choice of species and why the animal model(s)	We are using the lowest form of mammals possible in each case (mice, rats and rabbits). We are also using tried and tested models to test our hypothesis and so are eliminating any variables that may arise from the

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.	employment of non-validated models. When we subject animals to a procedure we will be informed by studies that have been carried out on cells as to the dose to use for each new procedure. We will dose single animals and observe the animal for unexpected and unpredictable effects before going on to dose a group of animals.

<table>
<tr><td>PROJECT 15</td><td>Development of novel agents for cancer therapy</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cancer, novel drugs</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[21]</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[22]</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>These studies are designed to identify optimal dosing regimens for novel and existing compounds based on the regulation of so called ‘biomarkers’. In these studies a ‘biomarker’ means a biological change which can be detected when a drug hits its target molecule. This approach of measuring biomarkers and using this to decide how much drug to dose and how often is the foundation of effective in vivo testing of compounds in mouse models of cancer, and ensures the efficient and effective use of animals in future studies.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the</td><td>These studies should form the basis of future testing for compounds in clinical studies in patients. Biomarker approaches validated in these experiments will be translated into clinical measurements that can be used in patient studies.</td></tr>
</table>

project)?	This “translational biomarker” approach is critical to ensure that novel compounds are effectively tested in the clinic and their effects understood.
What species and approximate numbers of animals do you expect to use over what period of time?	Only mice will be used for these studies. Over a period of 5 years, we anticipate using a maximum of 2500 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?	Studies performed under this license will not exceed moderate levels of severity. Some experiments using novel compounds may involve multiple dosing which may occasionally lead to unexpected adverse clinical signs being observed. However, experiments will be designed to ensure that compound exposure is given in a stepwise manner to minimise the impact of unexpected toxicity. All animals will be humanely killed by a Schedule 1 method at the end of procedures.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In this programme of work testing in human cells will be used to extensively evaluate compounds in terms of efficacy, toxicity and likely bioavailability (i.e. exposure in animals). However, it is only in whole animals that the complexity and interplay between biological systems can demonstrate if a compound will be absorbed after dosing and have an effect on processes of cancer that are expected to translate into effectiveness in patients.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Studies with compounds will be performed using a robust and reproducible design including randomisation, suitable vehicle controls, consistent dosing, sampling and analysis methodology. This approach gives the best opportunity to generate clear decision-making data on compound effects and efficient progression into mouse models of cancer with minimal use of animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	This licence uses wildtype and immunocompromised mice that are no greater than moderate severity. It is essential that we use immunocompromised mice in order for non-mouse

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	cell lines to grow tumours successfully without rejection by the host. Wild-type mice (which have a normal immune system) may also be used in studies where the immune response is thought to play a key role. To minimise suffering, all mice on procedure will be constantly monitored and humanely killed when exhibiting signs of altered health status and/or tumour burden. All users will be fully trained in monitoring tumour development for each model and will be signed as competent prior to initiating their own *in vivo* studies. Our animal unit is proactive in environmental enrichment and provides fun tunnels and nesting materials in cages.

<table>
<tr><td>PROJECT 16</td><td colspan="3">Development of personalised anti-cancer strategies</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Cancer. Drugs. Translation. Biomarkers. Metastasis.</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[23]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[24]</td><td></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">The main objective of this project is to use mouse models of cancer to identify new drugs or combinations of drugs to treat patients. In order to make sure that the right patients are getting the right drugs, research will also be conducted to develop tests that can tell doctors if a patient is likely to benefit from the new drug before they take it or to tell the doctor quickly whether the drug is working. Finally, research will be carried out to better understand how cancer spreads around a mouse, so that new drugs can be developed to stop the spread of cancer.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be</td><td colspan="3">The research carried out in this project is likely to tell us whether new drugs will work in certain cancer patients. This would then allow doctors to</td></tr>
</table>

advanced or humans or animals could benefit from the project)?	test these drugs in patients in clinical trials, with the ultimate aim of finding drugs that can be approved and used to treat patients on a regular basis. Alternatively, the research might suggest that drugs will not work in patients, which will allow pharmaceutical companies and doctors to make an informed decision not to try the drugs in patients, allowing those patients to try a drug that has a better chance of working. The research should also allow us to better understand why some patient's cancers respond to a drug and others do not. From this, further research could identify new drugs that might work in those patients who do not respond to the current drugs.
What species and approximate numbers of animals do you expect to use over what period of time?	Studies will be performed in adult mice, using approximately 17,000 over a 5 year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Continuous improvement in husbandry and experimental procedures minimize actual or potential pain, suffering, distress or lasting harm. The expected adverse effects for treatment of mice with anti-cancer drugs are weight loss, a change in normal behaviour and a loss of condition of their fur. The mice will be closely monitored by trained and competent scientists when they are being given drugs to make sure that they do not go beyond a 'moderate' level of severity. In the unexpected event that they do suffer more than this they will be humanely euthanised by trained staff. At the end of the experiment, all 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	Cancer is a complicated disease, with cancer cells interacting with lots of other cells in the body, e.g. blood vessels. Drugs also interact with other cells in the body – the best example of this is a cancer patient's hair falling out when they are given a drug. Therefore, to get a true reflection about how a

	cancer and the patient is going to respond to a drug it is important to carry out the research in an animal and not in a test-tube. By doing this research in animals, it gives the best chance that the finding will be relevant when the drug is used in patients.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Before a large experiment is carried out a small experiment (pilot experiment) will be done, to make sure that the cancer model is growing properly and to estimate the least number of mice that need to be used for the full experiment to get reliable information. Using new technologies and techniques will allow the minimum number of animals to be used. For example, due to advances in blood analysis it is possible to analyse drug levels from small blood samples. Therefore, a small sample can be taken from the same animal at different times, rather than a different animal being used each time, reducing the 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.	The conditions under which experimental animals are kept are designed for the least possible disruption of natural behaviour and the highest possible quality of life. Continuous improvement in husbandry and experimental procedures minimize actual or potential pain, suffering, distress or lasting harm and/or improve animal welfare in situations where the use of animals is unavoidable. Experience with pharmacodynamics studies has helped refine treatment scheduling. When dosing animals on an individual basis, as their tumour volumes reach treatment size, the data collected could prove variable. A change in study design now sees treatments start at a defined time point post implantation, when the tumour volumes are between a specified size window. All animals are treated at the same time, and any mice with tumour growth kinetics outside of the threshold do not have to undergo any further procedures. The resulting data acquired has been more robust and reduced the number of animals that receive chemotherapy unnecessarily.

<table>
<tr><td>PROJECT 17</td><td colspan="3">Microenvironment signalling in cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Cancer, microenvironment, therapy</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 yrs</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[25]</td><td colspan="3"><table>
<tr><td>Basic research</td><td><u>Yes</u></td><td></td></tr>
<tr><td>Translational and applied research</td><td><u>Yes</u></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[26]</td><td></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">In the UK, cancer kills over 400 people per day (this means over 730,000 people over a period of 5 years). Modern cancer therapies very efficiently target the cancer cells within a tumour, but there are other non-cancer cells in the so called ‘tumour microenvironment’, which can help the cancer cells to resist the therapy. We want to understand how the complex ‘tumour microenvironment’ can communicate with the cancer cells.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">Understanding how the ‘tumour microenvironment’ communicates with cancer cells will allow the identification of drugs that can suppress the help that the ‘tumour microenvironment’ offers the cancer cells, and ultimately can improve the overall survival of patients receiving cancer therapies.</td></tr>
</table>

What species and approximate numbers of animals do you expect to use over what period of time?	We are planning to use approximately 700 mice over a period of 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The expected level of severity is moderate. Animals (including immunocompromised animals) will undergo surgery or receive injections, and appropriate anaesthetic drugs and drugs for pain relief will be given to the animals. Discomfort can occur from the growth of a tumour, including swelling of tissue covering the tumour, skin ulceration and weight loss. Laboured breathing may suggest the existence of tumour cell spread in the lungs. If any of those signs are observed, the mouse will immediately be sacrificed following a protocol for humane killing. At the end of each experiment all animals will be humanely sacrificed, so that we can perform all relevant analyses.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The 'tumour microenvironment' is extremely complex; it consists of other non-cancerous cell types, a connective tissue, a lymphatic system and blood supply. Although many researchers, including our group are currently developing more sophisticated cell culture systems that resemble certain components of these complex conditions, we are far from being able to reconstitute the full 'tumour' situation; which means that it is currently impossible to adequately study the 'tumour microenvironment' outside the body. If appropriate alternative non-animal models become available that allow replacing the use of animals, these will immediately be adopted. Before the start and throughout the project we will make sure that our research does not unnecessarily duplicate experiments.
2. Reduction Explain how you will assure	The number of animals to be used will be calculated with the advice from designated statisticians. The experiments are designed in a

the use of minimum numbers of animals	manner that will allow using one animal for a whole series of cell culture experiments in the lab. Furthermore, by combining the animal experiments with our cell culture experiments will allow using the minimum number of animals to achieve our objectives.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The mouse is the lowest vertebrate that offers a 'living body' situation relevant to human cancer and that can be manipulated in a manner that will allow producing meaningful data relevant for the treatment of human cancer. Due to the nature of cancer, unfortunately we cannot use an invertebrate, and even the use of other vertebrates such as zebrafish larvae is limited due to the lack of the formation of blood-borne secondary cancers (metastasis). As such, the mouse is the only available animal model to achieve the stated objectives. All chosen regulated procedures will be performed by highly trained staff, which ensures brevity of the procedure and the lowest level of discomfort. By responsibly considering the adverse effects associated with what will be done to the animals, mechanisms are in place to minimise these.

PROJECT 18	**Novel multifunctional phage for guided systemic cancer gene therapy**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals[27]
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Although originally conceived to treat inherited diseases, to date over 70% of clinical gene therapy trials are designed to treat cancer. Cancer is a major cause of morbidity and mortality in the world despite progress in conventional treatments. Cancer gene therapy is a promising strategy for the treatment of cancer; however, most clinical trials have failed. Indeed gene therapy has faced a major challenge which is the lack of this ideal vector that can deliver anticancer genes to tumours selectively and at therapeutic levels while sparing the healthy tissues, following intravenous administration of vectors into the blood circulation. Localized delivery of the therapeutic genes is necessary for proof-of-principle; however, real clinical benefit can only be realized with non-invasive intravenous administration, a route that is applicable for both localized and metastatic disease. Animal viruses have been mostly used to carry the therapeutic genes; however, these viruses have broad tropism for the healthy tissues leading to their accumulation in these tissues following intravenous administration, which prevent them from	

	reaching the tumours. Thus, it is crucial to develop vectors with potential to attain cancer through the systemic circulation. In our previous work, and under the expiring PPL70-7035, we have designed and developed a novel viral vector that showed successful targeting of anticancer genes to tumours following intravenous administration in tumour-bearing mice, without accumulation in the normal tissues. This novel vector is based on bacteriophage, which is a virus that infects bacteria only. Bacteriophages are safe and have long been given to adult and children over many years to treat bacterial infections. Bacteriophage doesn't infect human cells; thus by displaying, on bacteriophage, a ligand that binds and targets specific receptors in tumours, this vector was able to target the tumours following intravenous administration and deliver the anticancer genes. In this application, our objectives will be to assess targeted gene therapy by this novel bacteriophage vector against intracranial human glioblastoma established in mice. Glioblastoma is a lethal cancer and survival remains disappointing; it is, therefore, critical that we identify effective medical therapies against this cancer disease. Glioblastoma was one of the early targets of gene therapy but failure of clinical trials was mainly due to inefficacy of the vectors. It is important to mention that bacteriophage is known for its ability to cross the blood brain barrier.
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)?	Targeted systemic gene therapy would represent a major advance in the management of glioblastoma. If successful, our strategy is likely to lead to useful clinical applications related to glioblastoma gene therapy. Moreover, and given that our ligands could also target most cancers, these advances are likely to extend the potential of bacteriophage-guided gene therapy targeting to human cancers in general and mestastases. Indeed, our therapeutic approach is delivered intravenously, a clinically non-invasive route, applicable in both localized and metastatic tumours. It is noteworthy to mention that bacteriophage-guided gene therapy can rapidly enter clinical trials in cancer patients as bacteriophage has already been safely used for clinical applications in adult and children over many years. Phage vectors are highly amenable

	to production to GMP standard.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice. We expect to use a maximum of 600 animals over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The animals may experience the following adverse effects as a consequence of the large size tumours, with moderate level of severity: weight loss, motor dysfunction (falling over, ataxia, reduced mobility), dehydration, sunken flanks, seizures. The animals will be observed twice a day. Where any one of these signs is present in a single animal then it will be killed immediately by terminal anaesthesia plus perfusion or by a Schedule 1 method and any remaining animals observed closely for changes in their condition. At the end of the experiments, animals will be anaesthetised, perfused through the heart and tumours and other organs will be harvested.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	*In vitro* models do not approximate the clinical condition as well as animal models. For example, the intact brain consists of various interacting cell types which each have a major function in brain disease. Moreover, the brain has a blood supply and inflammatory/immune responses that also play pivotal roles in brain tumours. The blood brain barrier and the microenvironment of the brain cannot be duplicated by other methodologies. *In vivo* experiments to gain greater insight into the human condition are therefore crucial. We have attempted to use a three-dimensional (3D) tumour spheroid model to recapitulate features of tumour microregions. However, this 3D model imitates the avascular regions of tumours only, while in our project all treatments will be carried out through the intravenous administration. Moreover, we intend to assess the tumour targeting potential of our vector, which can only be investigated by comparing tumour homing of this targeted vector to its homing to the normal healthy tissues in individual animals.

2. Reduction Explain how you will assure the use of minimum numbers of animals	We will maximise the amount of data generated from a single experiment by combining imaging of the tumour-bearing mice with therapy. Indeed, all the implanted tumour cell lines will express the luciferase imaging gene that allows bioluminescent imaging (BLI) of the whole living tumour-bearing mice. Indeed, BLI allows tumour detection, will measure tumour viability and size, and monitor tumour response to therapy in individual animals. This strategy allowed us, in our previous studies and under our expiring project licence, to generate maximum amount of publishable data from each experiment, thus reducing the need to repeat experiments several times, and allowing the use of minimum numbers of animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the most commonly used species for the type of procedures detailed within this application. Mice have been, and continue to be used extensively in cancer research because they can be used to model human tumours. These rodents are relatively low order sentient animals (i.e., compared with non-human primates, cats, dogs, etc.,); however, these species are accepted by the scientific community as the standard models for the establishment of cancer models *in vivo* and for the assessment of the therapeutic efficacy of gene therapy vectors, which covers the kind of translational research we intend to carry out. We will take every measure possible to avoid unnecessary animal suffering. All the implanted tumour cell lines will express the luciferase imaging gene that allows BLI imaging of the whole living tumour-bearing mice. Thus, BLI will allow tumour detection, monitoring tumour response to therapy and generation of maximum amount of data allowing early termination of our experiments while the tumours haven't reached large size. This should minimise animal suffering that could result from large size tumours. Finally, as discussed in this application, objective#1 will provide the necessary information regarding the therapeutic anticancer gene candidate to be tested further in mice with intracranial tumours; thus we will

	use the minimum number of mice to achieve objective#2 and subsequently to minimise animal suffering that could result from intracranial tumours.

<table>
<tr><td>PROJECT 19</td><td colspan="3">Evaluation of anti-cancer drugs</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Cancer, drugs</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[28]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[29]</td><td>Yes</td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Cancer affects 1 in 3 people. Although there are already many treatments that can prolong a patient’s life, many patients will not live for another 5 years after cancer is diagnosed. For example, in the case of ovarian cancer, chemotherapy often increases the patient’s life expectancy, but many patients develop a form of the cancer that is resistant to further therapy. This means that these patients will live on average 2-3 after diagnosis. There is clearly a need for new treatments.</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</td><td colspan="3">We are conducting experiments in the laboratory to identify new anti-cancer drugs. We hope that these can one day be used to treat patients. It is relatively easy to kill cancer cells in the laboratory, but harder to</td></tr>
</table>

the project)?	do it in a patient because the drug may make the patient ill. So before the drugs can be given to large number of patients, they are usually tested in a relatively small number of patients in a "clinical trial" to see if the drugs kill the cancer cells without making the patients sick. However, this means giving a patient a drug that has perhaps never been given to a human before. To justify doing this, we have to have a reasonable expectation that the drugs will work in patients. We can only do this by testing the drug in animals.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 120 mice per year over a 5 year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The adverse effects of the drugs depend on the type of drugs we test. Sometimes we are testing drugs that have been used before and we know what adverse effects to expect. But many of the drugs are new and although they are designed to specifically target the cancer cells, they may have unexpected effects. This makes it hard to know what adverse effects might occur. To minimize the adverse effects on the animals, we will first test the drug on a small number of animals to identify a dose of drug that has only moderate adverse effects. The experiment will end when the tumours reach a relatively small size so that any stress to the animal is minimized. At the end of the experiment, the 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	We wish to find new drugs to treat cancer. We are testing both drugs that have been used to treat other diseases as well as drugs that are completely new. We need to gather

alternatives	sufficient information to convince doctors that the drugs which we will identify are likely to work in patients – it is not reasonable to give a patient who is already quite ill a new drug unless there is a reasonable likelihood that the drug will do more good than harm. To gather this information, we need to test the drugs using a method as close to real patients as possible. Although we can (and will) test the drugs in the laboratory, these types of studies are not sufficiently complex to model what happens in real patients. Animals are not a perfect model, but they are the closest model we have. Using computer simulations can help, but simulations are limited to testing things we already know about. Many drugs that we will test are completely new, or they are established drugs being used in a different way, so we don't know what will happen. We can't make a computer model of something we don't know about.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will design our experiments to minimize the numbers of animals used. Firstly, all the drugs which are tested in animals will f be tested in several different types of experiment in the laboratory to confirm they have the desired anti-cancer activity. We will also try to mimic in the laboratory the conditions in the body. If the drugs do not work in these studies, we will not test them animals. This "triage" process will minimize unnecessary testing. Secondly, we will conduct studies in the laboratory that form a "bridge" between the laboratory studies and the animal studies. In these we will try to estimate how often the drug needs to be given to the animals. When we move to testing the drug in animals, we will first conduct experiments with a relatively small number of animals to establish the

	correct dose of drug and the frequency at which is administered. Both these strategies will minimize the chance of an experiment having to be repeated because it was incorrectly designed. We will also use statistical principles to estimate the minimum numbers of animals we need to use to measure the effect of the drug.
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 the species least likely to suffer distress without compromising the likelihood of studies working. We will use special mice with a defective immune system to prevent them having a reaction against the tumour cells. This is the most commonly used species for this type of test. All of our studies have been reviewed to consider how to minimize costs to the animals. **Animals will be maintained in an environment that supports their normal behaviour** We have considered any likely adverse effects of the procedures we will use, and we will regularly monitor the animals to make sure that the animal are not distressed. We may use optical imaging to help us monitor growth of tumours. Staff will be trained to recognize signs of distress and how to deal with this situation.

<table>
<tr><td>PROJECT 20</td><td>Epithelial-Stromal Interaction in Cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cancer, Stroma, Epithelium, Breast, Lung</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td></td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Cancers consist not just of malignant tumour cells but also a number of other cells that are not malignant. These include cells that provide nutrition and growth factors to the tumours and which form an essential support to the tumour as it progresses to be able to escape from the local environment to a distant site. This process is known as metastasis and is the primary cause of death among cancer patients. Unfortunately overall survival for these patients with metastasis has not changed for twenty years. This indicates that the current medicines are ineffective for treatment of metastatic disease and this demands development of new medicines in order to effectively cure the disease.

The natural history of cancer is that they progress from benign (not life threatening) to malignant (life threatening) stages. Our and others studies have</td></tr>
</table>

	indicated that these transitions to the most dangerous stages of the diseases is speeded up by the normal cells in the tumour environment. Our previous research has indicated that killing these non-malignant cells in the tumour can inhibit tumour cell metastasis. Our experiments have mainly focussed upon the sub-populations of a type of blood cells called macrophages that reside within the tumour. In particular we have indicated that these tumour-associated macrophages are important for the spreading of cancer cells. At each step of this process macrophages provide support to the cancer cells. Consequently our general experimental plan is to use mouse models of cancer to define the molecules involved in the tumour cell-macrophage interaction and the relationship of these cells with other components of the tumour microenvironment. Thus while it is now established in the majority of pre-clinical mouse models that the tumour microenvironment regulates metastatic progression the actual basis of these effects still largely remain to be explained. This project aims to determine the molecular basis for these macrophage actions using genetic and imaging methods, which will indicate targets that may lead the development of novel therapeutics directed to the tumour microenvironment and may ultimately be used in humans. The goal of this animal based research therefore is to develop an understanding of the cellular mechanisms responsible for the development and spread of cancer in an attempt to define possible therapeutic strategies in humans. The potential benefits of this research are to provide the basis for cure of metastatic disease in a wide range of 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)?	Through these studies, we will be able to reveal the role of tumour surrounding normal cells (stromal cells) in tumour progression and metastasis. This project also allows us to identify stromal factors that affect cancer cell behaviour, anti-tumour immune responses, and efficiency of current therapies. Such information will provide novel strategies for targeting stromal cells to prevent tumour

	progression and metastasis. Comparison of stromal cell functions between healthy tissue and tumour tissue gives us essential information to generate more specific and therefore less toxic therapeutic strategies. Investigatiion of pro-tumorigenic functions of stromal cells will also provide important information for cancer researchers to develop novel prognostic markers to predict disease outcomes, diagnostic markers to follow disease progression and select optimal therapy to prevent cancer progression to the more deadly forms.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse 65,000 over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Both experimental and genetically engineered mouse (GEM) models of cancer develop benign to malignant tumours, which may cause moderate discomfort. However, their development is tightly controlled and localized to minimize the suffering. Furthermore, the tumour-bearing animals are regularly monitored for their clinical signs, and are euthanized before severe effects are observed. All surgery and injection methods are well established and we have enough experience to minimize infection and health problems that may arise from these methods. So far, intravital imaging is the only method to reveal tumour cell behaviour in the complex environment. To obtain clear images, we will insert a small window over the tumours developed under the skin or in the mammary gland, which will cause momentary pain to the mice and possibly to induce post surgical inflammation but only in rare case. However, pain is controlled by general anaesthesia and analgesics, and risk of infection is minimized by good surgical and aseptic techniques. The implantation site is monitored for signs of inflammation and infection, and antibiotics will be given if necessary. A small window is also inserted into pulmonary cavity to analyse tumour cell behaviour in the lung. This

	procedure is performed under terminal anaesthesia with close monitoring of the mouse's respiration and heartbeat. Therefore, animals will not feel any pain or discomfort. All the animals used in the experiments will be euthanized at the end point.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We use animal models in these experiments because tumour progression involves progressive genetic changes that cannot be modelled adequately by cells in culture. Furthermore, the complex tumour environment contains many different cell types whose interactions can only be found in vivo. For these reasons only animal models can be used. We chose mice as an experimental animal because cancer in these animals has been studied for many years giving us a good foundation for our experiments. Furthermore our group and others have carefully documented tumour progression in this species for several different tumour types. We have some mice in which human cancer cells or tissue fragments can be grown, which allows us to study them in a fashion that is not possible in culture.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To reduce numbers of mice, when possible, we will study cell interactions between different cell types *in vitro* using systems we have developed to study tumour cell invasion, escape into and out of the blood system. These multi-cellular cell culture models will be used to screen possible molecules involved in the macrophage promotion of metastasis before they are introduced into the more complex but more real in vivo environment. To find out effective therapeutic targets, our study requires various single and compound genetically engineered (GE) strains, which requires complicated breeding strategies. To minimize the number of animals involved we have now identified the most efficient breeding crosses to generate mice of the correct genotype. To reduce the required numbers of GE animals, we will transplant bone marrow or mammary gland from GE animals to wild type recipients because it can provide

	enough animals with same genetic mutations in blood cells or mammary gland without further breeding. We will also use non-invasive *in vivo* imaging to detect and measure internal tumours, which will greatly reduce the number of mice to be required. We ensure the experimental plans are designed using statistical principles to test our specific hypotheses and thereby only use the numbers of mice necessary. We will therefore use the minimum number of mice possible and this amount used for experiments to approximately 13,000 per year for a period of five years.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We use mouse tumour models that have been developed to mimic the progression of the disease in humans as much as possible to develop more effective therapeutics and prevention strategies of metastatic cancers. Our research group has developed many of these models and thus have vast experience of the clinical signs they will show, which allows us to avoid severe health problems in the animals during the experiments. Our experience and the data that we generate will enable us to understand tumour progression better as our studies progress, and therefore develop increasingly humane end points. We will ensure that all animals receive the highest standard of care, and animal suffering is kept to a minimum by close monitoring of tumour development We will use our refined genetic modification techniques to improve the welfare of our animals and well-designed experimental strategies to reduce the number of required animals. We also have developed methods to examine tumours in real life and time using non-invasive and non-terminal methods that limit the numbers of mice used. Furthermore, we continue to monitor technical advances and to innovate novel techniques in an attempt to reduce the impact of experimental cancer on the animals. If suitable models occur, we will immediately adopt them into our research strategy.

PROJECT 21	**Biology of relapsed childhood leukaemia**	
Key Words (max. 5 words)	Leukaemia, drug-resistance, relapse, bone marrow microenvironment	
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)	To understand the mechanism of relapsed childhood leukaemia, that is, why and how leukaemia returns even after chemotherapy	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Although -85% children with acute lymphoblastic leukaemia are now cured, the disease will return (relapse) in around 15% of children, with only 30-40% surviving. This makes relapsed ALL the fourth most common childhood cancer. As treatments are now given at maximum doses, with toxicity-related deaths matching the numbers of death from disease, it is important that new drugs are identified. The work outlined in this licence will contribute to a greater understanding of the biology of relapsed leukaemia	

	which will hopefully lead to the identification of novel, less damaging drugs resulting in fewer deaths from childhood leukaemia.
What species and approximate numbers of animals do you expect to use over what period of time?	We plan to use approximately 2,500 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?	We will be transplanting patient cells into the mice to generate a mouse model of relapsed leukaemia. The growth of these cells in the mice may cause ill health such as weight loss or a limp if the cells affect the central nervous system. Mice displaying signs of sickness will be very closely monitored for 24 hours and humanely culled if necessary. The severity of our studies is expected to be mild to moderate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Leukaemia cells reside in the bone marrow and we are interested in the relationship between these malignant cells and the surrounding healthy cells, known as the bone marrow 'microenvironment' The cells in the bone marrow can communicate with one another in different ways — by directly interacting with one another or by releasing 'messages' that can be taken up by neighbouring cells. In this way, we believe that a small group of leukaemic cells can be protected by the microenvironment from the effects of chemotherapy and contribute to a return of the disease (relapse). In addition, we have also shown that leukaeniia cells can release small, membrane bound sacks known as vesicles that can be taken up by a wide variety of cells. We believe that the contents of these vesicles allow signalling to neighbouring cells and that by releasing these, the leukaemic cell can 'persuade' the surrounding cells to create an environment that is favourable for its own survival. If we are to fully understand the mechanisms of chemoprotection and manipulation of the bone marrow to create a tumour-niche, experiments need to be performed in the context of a whole, intact blood

	(haematopoietic) system: the use of cell culture work in the laboratory (in vitro) lacks the cell-to-cell interactions and microenvironmental contributions that would be present in a mouse (in vivo) setting. Only in this way can we develop meaningful drugs to overcome relapsed leukaemia.
2. Reduction Explain how you will assure the use of minimum numbers of animals	When new drugs or cellular material are to be used in the mice, pilot studies will be carried on small numbers of mice (say, 3) first. When mice are culled, tissues such as spleen, bone marrow and liver are to be harvested for use in other areas of the project so that mice are not used unnecessarily. Statistical advice will be sought from our own statistician to ensure that the lowest numbers of animals will be used that can generate meaningful data.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We have chosen to use mice in our study as they are the least sensitive animal model of human haematopoiesis (production of blood cells). They are genetically very similar to human beings, with 99% of human genes having a murine version. Their short life cycle and rapid reproduction rate allows disease progression to be easily followed. Murine and human haematopoietic systems are also very similar unlike those of the less sensitive non-mammalian species such as the zebra fish. For example, the zebra fish possesses a different type of red blood cell, has no platelets and uses the kidney instead of the bone marrow as a site for making cells of the blood/haematopoietic system. The techniques that we are proposing to employ will involve the least distress to the animal whilst still generating meaningful data. For example, the chosen dose of irradiation will suppress production of certain cells in the bone marrow, but will cause no long term adverse effects.

	Pilot studies will always be performed for drugs not used previously. Daily health monitoring of experimental animals will allow any suffering caused by protocols to be kept to a minimum. Twice-weekly weighing will be increased to 48 hours if required Cancer cells will be detected by imaging the mice rather than feeling (palpating) the body of the mouse. In this way, earlier detection of disease spread into areas such as the liver and the spleen will be possible.

PROJECT 22	**Immunisation of Rodents**	
Key Words (max. 5 words)	Immunise antigens antibodies	
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)	To provide a service of rodent immunisation for the purpose of production of novel antibodies. The antibodies will be used to detect and/or quantify the corresponding antigens in biological tests carried out for basic and applied research.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The potential benefits are the application of the antibodies produced to our improved understanding of biology, the establishment of permanent sources of valuable reagents which can reduce the need for animals used for antibody generation. Collaborations will allow other scientists, who do not have experience in this technology, access to antibodies for use in their own project work.	
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse: 340 Rat: 60 Over 5 years	
In the context of what you propose to do to the animals,	Irritation, chronic inflammation and the formation of lumps composed of immune cells can occur.	

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	However, the immunisation process and the routes of antigen administration to be used in this licence are not expected to result in ill health in the animals. Severity: mild All animals will be culled humanely by a Schedule 1 method at the end of a project
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Only complete animals contain the complex immune system that is required to generate specific, high affinity antibodies. While non-animal alternatives exist, these techniques produce incomplete, low affinity antibody fragments. Also, it is impossible to make antibodies against certain materials using non-animal alternatives.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The minimum number of animals required to ensure a successful outcome has been determined from work carried out on previous licences. However, methodologies will be reviewed over the life of the licence and in conjunction with discussions with other scientists working in the field of immunology may allow animal numbers to be reduced without affecting the rate of success.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Immunisation procedures in rodents have been carried out for over a century and are well characterised. Rodents are short lived, have rapid generation times and are easier to maintain than larger animals. The antibody producing spleen cells can be isolated so an infinite source of antibody can be established. Animals will be housed in a dedicated barrier unit where their welfare and health status will be monitored daily by trained staff. All protocols will be reviewed by a local animal ethics committee. Only trained technicians who have permission to carry out the procedures on their personal licences will complete the animal work. Finally, the routes of administration of the antigen do not cause adverse effects to animal health while producing strong immune responses.