Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 26

Project Titles and key words

- Development of Imaging Probes

  Imaging, Cancer therapy

- Investigation and modulation of cell migration

  Inflammatory bowel disease, breast cancer, inflammation, therapeutics, immunology

- In-vivo behaviour of progenitor cells

- Cortical and sub-cortical motor control

  Cortex; spinal cord; reticular formation

- Equine vaccine safety and efficacy

  Equine, Vaccine, Infectious Diseases

- Development and use of an in vitro method for studying EHV-1

  Equine herpesvirus in vitro model

- Equine influenza surveillance and transmission

  Influenza, transmission, pathogenicity, horses

- Magnetic Resonance Studies of Cancer and Cancer Metabolism

  MR imaging, MR spectroscopy, tumour, stroma, metabolism

- Tumour Implantation for Subsequent Treatment Screening

  Tumour, Implantation

- Tumour, Implantation

  Imprinted genes, pregnancy, metabolism, behaviour

- Studies of experimental small ruminant TSE

  TSE, scrapie, sheep

Development of Imaging Probes
Imaging, Cancer therapy,

- Summarise your project (1-2 sentences)

This project aims to develop new radioactive compounds that can be used to measure specific biological properties of cancer using an imaging technique called Positron Emission Tomography (PET). Knowing the properties of a patient's cancer will help identify if they might benefit from a particular therapy, and seeing how these properties change after treatment can help decide if treatment is working much earlier than other methods.

- 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.

Cancer is a leading cause of death in the west, and the successful treatment of this disease is currently an unmet clinical need. In the last decade new therapies targeted at specific molecules have proved successful in patients whose tumours rely on the target; however there is still the need to show that the drug is having an effect as early as possible to avoid unnecessary or ineffective therapy. Properties of cancer that can be measured to tell us something about it are often called 'biomarkers', and this licence aims to develop new imaging biomarkers to help guide cancer therapy.

Nuclear imaging is a technique where a radioactively labelled compounds injected into the body binds to or are taken up by cancer cells, and is detected using a tomographic scanner to generate a 3D image of where the probe is localised. There are two types of scanner, single photon (SPECT) and positron (PET), depending on the type of radioactivity used to label the probe, and both are used in the management of cancer. Although tracers for several major targets/cellular processes do exist, there is also scope for the development of novel probes with improved properties to study this biology.

As well as application in man, nuclear imaging probes can be used to study the progression and therapy response in animal cancer models over time. This results in the use of fewer animals, and better quality data as each animal can be used as its own control.

- Outline the general project plan.

Candidate probes will be tested in cell-based (*in vitro*) systems to test their specificity. Successful probes will be injected into animals to see how they distribute in the body, how they are metabolised and whether they can report on the target of interest. If successful, they will then be injected into mouse cancer models to see whether they can measure the responses of these tumours to cancer therapy.

- 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.

For biodistribution and metabolite studies, animals will be injected with a radioactive probe (either awake or under anaesthesia) and sacrificed at later timepoints. Blood may also be repeatedly sampled. There are no adverse effects expected for imaging with radioisotopes, and anaesthesia regimes used will be well established with the

physiological state of the animal monitored at all times. Blood sampling will be limited to 7.5% of total blood volume to avoid negative physiological effects, and will be terminal. Where necessary, food will be withdrawn to improve radiotracer uptake.

For studies in tumour bearing animals, immunocompromised mice will be inoculated with human cancer cells/tumour material to generate tumours for analysis. These will then be imaged, then subject to therapy and imaged again at one or more timepoints to assess the relationship of the imaging readout and response. Expected adverse events are the same as above, possible events such as infection in immunocompromised animals and weight loss resulting from tumour implantation will be avoided by the use of individually vented cages and regular animal monitoring. Only well-established cancer therapies will be used at previously reported doses to avoid toxicity.

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

The goal of this project is to develop new ways to measure the properties of cancer using imaging probes. If successful, measuring these properties will allow us to select patients for targeted therapy, as to see more quickly whether patients undergoing therapy are responding. Probes will also be used to study of animal models of cancer over time, allowing fewer numbers to be used.

- 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.

Over the course of the license, around 1000 mice will be used. Mice have been chosen as they allow the growth of a range of tumour models that best reflect the clinical situation in man, and are the species with the lowest 'neurophysiological sensitivity' in which such well-characterised models exist. There are many studies comparing data between mice and man that show the data gained from mice is 'translatable', i.e. can be used to base clinical trials on.
Numbers of animals are kept as low as possible to give meaningful results by using statistical techniques and guidelines on animal research.

- 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.

For novel probes, full cell-based analyses will be carried out before animal work. However, tests on cells cannot tell us if a new probe will be able to report on a target in a whole organism and so animal work is necessary. Animal studies are also needed before trials in man can take place, and so Replacement is therefore not possible. To see if probes can report on cancer treatment response, animal models must be used as it is not yet possible to model the very complex tumour environment and how this may change in response to treatment in any other way.

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

All protocols will be carried out in accordance with the UKCCCR guidelines on the welfare of animals in cancer research.

Investigation and modulation of cell migration
Inflammatory bowel disease, breast cancer, inflammation, therapeutics, immunology

- Summarise your project (1-2 sentences)

We aim to identify the biological pathways responsible for cellular movement during inflammation and cancer, and to identify methods of preventing or reversing this process in animal models that are applicable to the human immune system, specifically the conditions of inflammatory bowel disease and breast cancer.

- 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.

Inflammatory conditions and cancer are characterised by the abnormal movement of cells within the body. Recent scientific advances have led to the introduction of human-specific biological therapies for inflammatory and malignant diseases that have had some success in controlling this cellular movement. However as these therapies are specific to the human immune system, new human-relevant animal models are required to allow future discovery of targets for disease therapy, and also to test the safety of new medicines prior to safe human use.

- Outline the general project plan.

Our research project will utilise mice. We have recently developed a method of transplanting human white blood cells into mice that lack an immune system. We aim to use this model, and other specifically defined strains of mice to study and modify common immune pathways responsible for cell migration. We will also refine existing models of breast cancer and inflammatory bowel disease to study disease-specific biological pathways, in a bid to identify and test new targets for treating these debilitating conditions.

- 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.

General mechanisms of cell movement and the human diseases of inflammatory bowel disease and breast cancer will be simulated in the mice. Medications may be used to limit or reverse these conditions. The applicant has substantial expertise in the models outlined in this application, many of which have been developed as a result of previous project licences. This experience has allowed us to minimise the risk of significant adverse events, which are not expected in the experiments outlined in this application. Furthermore this experience minimises the necessity for animal usage in pilot experiments, and maximises the translational value that can be obtained from this work.

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

We believe that the results of our research could lead to an improved understanding of the factors influencing cellular migration in human disease, and that the development of human specific models of disease could lead to a reduction or replacement of higher order animals required for experimentation in other laboratories around the world.

- 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 expect to use an average of 120 mice per year over the course of this 5 year project. These will be a combination of typical form 'wildtype' mice, as well as some genetically modified mice, used specifically to study certain aspects of the immune system. This makes the information learned from these experiments of most relevance to human disease and the development of new treatments.

Statistical techniques have been used to ensure we use only the minimum number of mice for each stage of the experimental procedure.

- 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.

Many experiments will utilise mice that receive a transplant with human white blood cells in order to maximise the clinical implications learned from these experiments. New models for inflammatory bowel disease and breast cancer will be derived, improving upon existing animal models, many of which have limited applicability to human disease and the development of new medications.

We aim to generate disease that is relatively mild and therefore suitable for longitudinal monitoring. In the inflammatory bowel disease models we will monitor animals sequentially using colonoscopy and non-lethal intestinal biopsy to observe the generation and treatment of gut inflammation. We believe this non-lethal monitoring will lead to a 3-4 fold reduction in the number of mice required for the model.

Whilst non animal methods of experimentation cannot be used to answer the questions posed in this project, as they lack the biological specificity needed to study such complex immune processes, cells in a dish will be used to help select compounds for investigation.

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

Our group has been carrying out research in this field for over 25 years. We have used this experience, and that of the worldwide scientific community to ensure the designed protocols consider animal welfare, and minimise suffering. Appropriate analgesia and anaesthesia is planned for each relevant model, and our experience with these models and techniques suggests that significant adverse events should not be anticipated.

In-vivo behaviour of progenitor cells

- Summarise your project (1-2 sentences)

We want to make blood progenitor cells in the laboratory because these can be used to replace faulty cells in the bone marrow of leukaemia sufferers. Also, we want to understand how ageing affects the properties of blood progenitor cells normally present in the bone marrow, however this means we need to test the laboratory made cells in whole animals to see where they go in the body and how well they work over prolonged time periods

- 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.

  Bone marrow transplantation is used to treat leukaemia. This works because blood progenitor cells are present in the bone marrow and these can produce any type of cell normally found in the blood but it is difficult to get these cells from adult patients. We can make pluripotent stem cells (PSC) from the patients skin and subsequently turn those PSC into blood progenitor cells but we need to be sure that such “laboratory made” blood progenitor cells are able to do the same job as their natural counterparts before they can be applied to treating human diseases.

- Outline the general project plan.

We will inject the “laboratory made” blood progenitor cells into mice whose immune systems don't work so they won’t be able to recognise and reject the “foreign” cells. Moreover, we will knock out the existing bone marrow of the mice using radiation so most of the blood they make will be derived from the “laboratory made” blood progenitor cells.

- 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.

The most likely cause of adverse effects is the radiation treatment. From our own experience and over 30 years of research in this field by others, radiation doses are carefully controlled and mouse numbers kept to a minimum to achieve significant results whilst minimising adverse effects. In addition, irradiated mice will be carefully inspected at least twice a day for 4 weeks post-irradiation and will receive antibiotics to prevent infection whilst they regenerate a blood system.

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

This project will increase our understanding of laboratory-derived progenitor cells and how they behave following transplantation into a whole animal. Furthermore, we hope to develop new and improved ways to increase the number of progenitor cells that can be produced in the laboratory. Consequently, this project represents a vital step towards the use of progenitor cells therapeutically.

- 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.

No more than 2,600 mice (including 1,000 immunodeficient mice) will be used in this project. The use of reprogrammable mice is essential for the study of progenitor cell ageing since we need to be able to convert aged progenitor cells back to pluripotent stem cells with high efficiency.
NSG mice are essential for functional engraftment of progenitor cells and NOD/SCID mice are needed for the assessment of stem cell pluripotency using the teratoma formation assay. We will ensure that the minimum numbers of animals are sued as follows; The use of anaesthesia to immobilise mice prior to irradiation will also minimise variation in dosimetry and thus reduce the size of groups required. The use also of genetically defined inbred strains of mice reduces the need for large-scale experiments designed to eliminate genetic variation.

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.

Mice are needed for this project firstly because they are sufficiently similar to humans to generate meaningful data that can be translated to human development and disease and secondly because mouse genetics has advanced to such an extent that mice provide an extremely powerful way of analysing the role of key genes in haematopoietic development – a process that we cannot effectively mimic *in vitro*

That notwithstanding, large sections of our programme of investigation can be performed using in vitro techniques thereby reducing the requirement for animal experiments. For example, substantial amounts of data concerning the functional analysis of HSC can be obtained using in vitro colony assays. This will contribute to investigations of the functions of PSC derived HSC and HSC ageing. Similarly, we will perform preliminary screening for candidate genes that can influence PSC differentiation to HSC *in vitro*. Only when strong candidates emerge from this process, will transplantation of genetically modified HSC be performed.

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

I have designed the protocols to minimise distress of the experimental animals. Where invasive procedures will be carried out, we will use anaesthetics and analgesics as appropriate. Any loss of condition as shown in Appendix A will indicate removal from the procedure and killing by Schedule 1 method. The use of prophylactic antibiotics during myeloablative protocols will reduce adventitious infection. We will keep the immunocompromised recipients on isolators to avoid loss of viability due to infections. Where possible, the experiments will be carried out in collaboration with groups that have a large body of expertise with a particular model.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Cortical and sub-cortical motor control</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cortex; spinal cord; reticular formation</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[1]</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[2]</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>This project has the overall aim of improving our understanding of the different brain and spinal cord centres involved in the control of movement, and translating the knowledge into improvements in therapy for patients recovering from injury, such as after stroke or spinal cord injury.

Specifically, we aim to understand the relative contributions of different parts of the nervous system to movement control in healthy animals, and how information is processed within each neural centre. We will then map how the surviving centres change after damage. We also aim to understand the processes which can change neural connections within these circuits, and to use this knowledge to devise stimulus protocols which can modify connections.</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>Stroke is currently the leading cause of disability in the UK. There are around 150,000 new strokes annually, one quarter in individuals aged under 65. The UK has 1.2m stroke survivors, around half of whom live with a disability that affects their everyday life. Total care costs for stroke in the UK are estimated at £8.2 billion (all figures taken from The Stroke Association). Therapeutic options for improved rehabilitation are limited, especially for hand function – one reason for this is a poor understanding of the scientific basis for motor control, and the processes underlying its recovery after insult. The information gained by this project will allow us to devise principled new strategies for therapy to improve rehabilitation. If this leads to</td></tr>
</table>

[1] Delete Yes or No as appropriate.
[2] At least one additional purpose must be selected with this option.

	even small improvements in function, it will translate into major social and economic impact.
What species and approximate numbers of animals do you expect to use over what period of time?	40 macaque monkeys over 5 years 250 rats over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Monkeys will be trained to accept some restraint (a neck collar), and to perform a behavioural task. They are motivated to perform the task by having restricted access to food, and occasionally fluid; food and fluid rewards are then given for correct task performance. After training is complete, they are surgically implanted with a headpiece to allow head stabilisation and electrodes to record muscle activity from the forelimb. Recordings will then be made from the central nervous system in the conscious state, whilst the animal performs the task. The most common adverse effects are associated with wound infections associated with the chronic implants. In a small proportion of animals, focal surgical lesions will be made on one side of the brain. In the days immediately following, these animals may need nursing help with feeding due to impaired movement ability. However, as in human stroke patients with small lesions they often show a rapid recovery. Rats may be prepared for recording by a surgery to inject neural tracers or novel genetic material, after which they are allowed to survive for a few weeks. Subsequent experiments involve terminal anaesthesia, and then making electrophysiological recordings or removing brain samples for analysis in vitro. Recovery from the initial surgery is unlikely to show adverse effects, and no adverse effects can occur in the final terminal procedure. The macaque experiments will have moderate severity, although the licence limit of ‘severe’ may be reached for short periods in some animals associated with the period immediately after a lesion. Rat experiments will be of moderate severity. At the end of experiments, all animals are humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This project investigates the complex interplay of brain circuits in different regions, and as such must be carried out in intact organisms. The laboratory does run a substantial programme of experiments

	in healthy human volunteers and patients; however, these can only produce indirect data. Detailed understanding at the level of single neurons and their connections can only be achieved using the invasive approaches possible in animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use sophisticated multi-electrode recording methods, which ensure that the maximum of data is gathered from each animal. Experiments in awake monkeys often yield sufficient data for publication from just two animals. Experiments under terminal anaesthesia use advanced anaesthetic methods to maintain the animals in good condition for extended periods (around 70 hours for macaques); this again enables us to gather extensive datasets from each animal.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Some basic circuit properties can be investigated in rats. However, the neural centres and connections controlling movement differ in key aspects in primates compared with non-primate species. To ensure that our results are directly applicable to human patients, we must use old world primates such as macaques. Our techniques have been refined over many years, and we continually seek to improve them. All recovery surgeries are carried out under full aseptic conditions, with advanced anaesthetic regimes which produce rapid and uneventful recovery. Full programmes of post-operative pain management are in place.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Equine vaccine safety and efficacy.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Equine, Vaccine, Infectious Diseases</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)[3]</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>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[4]</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The horse is susceptible to a wide range of diseases that can have a significant impact on its health and welfare. Vaccination is essential to prevent or limit their spread. The purpose of this project is to determine the safety and/or efficacy of vaccines and/or therapies specifically designed against equine diseases, such as that caused by equine influenza virus. Subsequently, vaccines and/or therapies will be improved (e.g. vaccine strain update) in order to enhance performance and protection.</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>This work is expected to provide a better understanding of the efficacy and mechanisms of action of vaccines and/or therapeutic procedures. In the case of the update or improvement of vaccine currently commercialised, this work will provide the necessary information for study sponsors and vaccine manufacturers to complete their regulatory requirements. Subsequent improvement of vaccines and/or therapy will result in reduction of the disease burden of horses and protection against potentially fatal diseases.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Most of the vaccine studies conducted under this project will follow the European Pharmacopoeia monographs, which usually recommend 10 animals per treatment group in order to obtain optimal information. A typical study will contain 1 or 2 treatment groups. Around 835 horses will be used over this five year project. This licence covers the inoculation of embryonated hens' eggs with equine influenza virus but the use of this procedure is not currently envisaged because the plan is to harvest</td></tr>
</table>

[3] Delete Yes or No as appropriate.
[4] At least one additional purpose must be selected with this option.

	virus before the eggs would become protected under the Act.
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?	No significant adverse effects on horses are expected after immunisation with the vaccines to be used during this project. However, the challenge infections required for efficacy studies do induce transient respiratory signs of disease in unprotected animals, which subside a few days after infection. The vast majority of immunised animals will be clinically protected and not become sick. To minimise adverse effects or complications, all animals will be monitored regularly and treated if necessary. Wherever feasible and appropriate animals are rehomed at the end of studies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To date, there is no *in vitro* model able to model such complex immune mechanisms. Therefore it is not possible to evaluate equine vaccines without using animals. When possible, archived samples will be used for optimisation of *in vitro* assays.
2. Reduction Explain how you will assure the use of minimum numbers of animals	A large amount of data and knowledge are available to inform the appropriate number of animals to use during an equine vaccination/therapy study. The number of animals to be used in each group is discussed with a statistician and optimised to maximise chances of identifying statistically significant results. When the study may be used for regulatory and/or authorisation purposes, the European monographs are consulted, when available. These documents provide a clear indication of the numbers of animals required.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	There are no reliable alternative animal models for evaluation of equine vaccines and the European Pharmacopoeia monograph for Equine Influenza vaccine requires testing vaccine efficiency in the natural host. Therefore, most of the studies that will be conducted under this project licence will require the use of a natural host model. The handlers that monitor the ponies know each individual animal well and use daily temperatures, clinical signs and behaviour to monitor their health and welfare. Environmental enrichment strategies are used to encourage normal behaviour.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Development and use of an in vitro method for studying EHV-1</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Equine herpesvirus in vitro model</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[5]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
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<tr><td>Preservation of species</td><td></td><td>No</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[6]</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>EHV-1 is a major pathogen of horses worldwide, causing respiratory disease, abortion, neonatal foal death and neurological disease. Outbreaks on stud farms in unvaccinated pregnant mares can lead to extremely high rates of late gestation abortion, or so called ‘abortion storms’ and neurological EHV-1 outbreaks are occurring more frequently in North America and Europe.

After initial infection in the upper respiratory tract epithelium, a highly cell-associated viraemia ensues. EHV-1-infected blood cells spread the virus to endothelial cells lining the blood vessels of the pregnant uterus and the central nervous system where it can cause most pathology. Why this is the case remains unclear. Such infection of endothelial cells can lead to inflammation in the venules and arterioles leading to blood clot formation and subsequent disruption of the blood flow that can result in tissue damage.

Commercially available vaccines against EHV-1, based on inactivated virus induce high levels of antibody and are able to reduce the levels of virus shed from infected horses. However, these vaccines do not prevent viraemia and do not protect against abortions and neurological disease, which are a major problem for the horse population in the UK. Consequently, a key event to control remains the infection of endothelial cells by virus-infected PBMC. In order to design improved vaccines or novel drugs to prevent EHV-1 associated disease, it</td></tr>
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[5] Delete Yes or No as appropriate.
[6] At least one additional purpose must be selected with this option.

	is essential to expand our knowledge about how EHV-1 spreads from peripheral blood mononuclear cells to endothelial cells and why endothelial cells in the brain or in the placenta and uterus of pregnant mares appear to be specifically susceptible to EHV-1 mediated pathology.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	*There is a current lack of understanding of how EHV-1 initially infects the respiratory epithelium and subsequently enters the blood stream which can result in infection of endothelial cells causing the serious clinical signs of abortion or neurological disease. Data obtained from this project will help us understand this EHV-1 disease process by identifying both host and viral proteins involved in this process. These proteins can then be targeted with drugs or vaccines for the benefit of the horse.*
What species and approximate numbers of animals do you expect to use over what period of time?	We will use up to 10 Welsh mountain ponies over the lifetime of 5-year project, allowing for the removal of animals from the licence based on advice from the veterinary surgeon or equine NACWO. We envisage no more than 2 animals being on the licence at any one 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?	Blood sampling is the only procedure that will be authorised. In order to keep any suffering to an absolute minimum, experienced staff will be used to take the blood samples. Adverse effects of repeated blood sampling are not expected and therefore the severity limit will be set to mild. Animals will be either re-used on another project or re-homed at the end of the program.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	EHV-1 has been shown to be highly cell associated during viraemia in the horse. Different blood cells circulating systemically have varying susceptibilities to EHV-1 infection, and can shed different amounts of infectious virus. Even the expression of specific viral antigens on the surface of different blood cell types varies. Existing cell lines cannot adequately model the plethora of cell types present in blood. Therefore in order to obtain the most accurate model and therefore most satisfactory scientific results, horse blood must be used. There are no suitable alternatives currently available.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The design of all animal experiments is subject to rigorous review by a 3Rs committee and then by an ethical review committee. This includes a requirement for cost-benefit analysis, justification for the use of animals and the number per experimental group. Development of this in vitro model will require the use of blood samples from no

	more than 2 individual ponies at any one time to allow for the minimum biological replicate. PBMCs will be tested for their response to specific stimuli and suitability for use in the model. Once appropriate cells have been identified and characterised, experiments will be conducted to determine whether cells can be stored in a frozen state for future use thereby reducing the number of bleeds required for a specific set of experiments.
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 Welsh mountain pony is a readily available natural host for EHV-1. The use of Welsh mountain pony blood in this project will permit the determination of host and viral factors important for pathogenicity and transmission in the natural host. The fundamental research being addressed by the programme will contribute to the welfare of horses in the long term. The one protocol on the licence is blood sampling. This will be undertaken by experienced staff on site thereby reducing the amount of stress suffered by the animals. Extensive experience of animal handling and regulated procedures suggests that adverse effects of repeated blood sampling are very rare and generally the more the ponies are handled, the more familiar they become with the routine and suffer less stress as a result. The ponies are kept at grass in large groups in grass paddocks with freedom to roam with shelter if they require.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Equine influenza surveillance and transmission</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Influenza, transmission, pathogenicity, horses</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)[7]</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[8]</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>Despite mandatory vaccination for competition horses in some countries, equine influenza remains a global threat to the racing and breeding industries. Infection spreads rapidly in unprotected horses, as shown by the outbreaks in Australia and Japan in 2007 and South America during 2012. These outbreaks affected thousands of horses, with significant economic and social impact.

Like other influenza A viruses, equine influenza viruses undergo antigenic drift, due to the gradual accumulation of changes in their surface proteins. Eventually, field strains change so much that vaccines stop working. To avoid mass breakdown of vaccination, the OIE (World Organisation for Animal Health) recommends suitable strains for inclusion in commercial vaccines. To decide whether strains need to be updated or not, current vaccine strains need to be tested against recent field isolates from around the World to determine whether they are likely to be effective or not. Detailed antigenic characterisation of recent strains also needs to be carried out to monitor changes occurring in the field. This horizon scanning approach gives an early warning system for major changes in field strains, with the hope that this will allow vaccines to be updated rapidly should the need arise.

Equine influenza viruses can sometimes also infect dogs and have become adapted to transmit</td></tr>
</table>

[7] Delete Yes or No as appropriate.
[8] At least one additional purpose must be selected with this option.

	efficiently between dogs in the USA. It is not clear yet how much the virus needs to change in order to infect and then transmit in a new host, but studies on individual virus proteins can help scientists determine which factors are important for the cross species transmission between species. Such studies have implication for other animals, including dogs, humans and seals.
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)?	Timely recommendation of new vaccine strains for equine influenza by the OIE, providing the information needed to update commercial vaccines. This in turn will reduce virus shedding by vaccinated horses, thus reducing the likelihood of large scale vaccine breakdown. Identification of new markers for cross-species transmission will inform basic research, with the potential for improved therapeutics and vaccines for humans and animals.
What species and approximate numbers of animals do you expect to use over what period of time?	The likely numbers of each species of animal to be used over a 5 year period are: (i) Mice – up to 1600 (ii) Ferrets – up to 20 (iii) Rabbits – up to 10 (iv) Welsh mountain ponies – up to 114 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?	Mice will be challenged with small volumes of equine influenza virus by the respiratory route, which is unlikely to cause lasting harm but may cause temporary stress due to being handled. Some mice may be challenged by the natural route, by mixing them with previously infected mice, which is likely to be less stressful as there will be no need for handling them. For vaccine studies, mice will also be immunised prior to virus challenge. This will involve additional handling and brief discomfort from injection, however very finely gauged needles are used for this procedure and it is unlikely to cause lasting harm. Mice will be euthanased at the end of each protocol. Ferrets will also be challenged with small volumes of equine influenza virus by the respiratory route, which is unlikely to cause lasting harm. They may experience very mild clinical signs, including occasional sneezing. Ferrets will be sedated prior to blood sampling, this procedure should therefore involve minimal stress. However they will need time to recover from the sedative. Ferrets will be euthanased at the end of each protocol.

	Rabbits will be immunised with small volumes of inactivated virus or proteins. They may be immunised several times, which is unlikely to cause lasting harm but may cause transient distress and discomfort from injection site reactions. Rabbits will be euthanased at the end of each protocol if a large blood sample is needed, where possible rabbits will be rehomed. Welsh mountain ponies will be challenged with equine influenza virus either by aerosol or the natural route, by mixing with infected animals. Unvaccinated animals are expected to develop typical clinical signs of influenza, including coughing, loss of appetite and raised temperature. Clinical signs typically last for 2 to 5 days and the ponies usually make a full recovery. Vaccinated ponies usually experience very mild clinical signs or asymptomatic infection. Ponies may be re-used under another project licence. Whenever possible, they will be rehomed after the completion of experimental procedures. The severity is not expected to exceed moderate for any procedure on this licence. The majority of the work will not exceed mild severity.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Antigenic characterisation of viruses, which is necessary for the process of vaccine strain selection, is dependent upon the generation of antibodies in another animal species. We primarily use ferrets for this, occasionally we also raise equine sera. To determine whether existing vaccine strains protect against new viruses, vaccines need to be tested in an animal model to show whether they protect against virus challenge or not. This is because influenza virus vaccines protect due to the induction of antibodies and/or cell mediated immunity in the host. There is no suitable in vitro model for this type of work. Wherever possible we use a mouse model to test vaccine strains, occasionally it is necessary to test them in the natural host for the infections – in this case, ponies. Many in vitro experiments carried out worldwide are dependent upon reagents that are generated in animals. In a particular, antibodies are used routinely to identify specific proteins. For our in vitro studies, in the first instance, we will attempt to use antibodies that have already been raised against other influenza viruses, however if these do not react well with equine influenza virus proteins we

	will need to raise sera in rabbits for this purpose.
2. Reduction Explain how you will assure the use of minimum numbers of animals	For raising antisera in ferrets and rabbits, two animals will be used for each experiment, for companionship purposes. This will ensure adequate sera can be collected to last several years whilst minimising stress to the animals. To keep the number of mice required to a minimum, pilot experiments will be conducted to calculate the infectious dose of the viruses used prior to carrying out full scale cross protection experiments. This will ensure that an adequate dose of virus is used and should eliminate the need for repeat experiments. For studies involving ponies, power calculations will be used to minimize the number of animals needed whilst maximising the probability of experimental success. We have many years experience in this area and will draw on data from previous experiments to aid the design of new studies, under guidance from epidemiologists and statisticians
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.	Ferrets are the model of choice for raising antisera for influenza viruses because they are readily infected by many different influenza A viruses and raise a strong and specific antibody response. Ferret sera have been used successfully for antigenic characterisation of equine influenza viruses for may years. A small number of equine sera will also be generated, usually only when there is a very large antigenic difference between virus strains. Equine sera do not discriminate as readily between virus isolates, but are still of use as they can be used to confirm that antigenic differences highlighted by the ferret or mouse model are also detected in the natural host. Under our previous project licence, the mouse model proved effective at discriminating between strains of EIV. It can therefore be used instead of ponies for an early warning system to determine whether vaccine strain recommendations should be updated or not. The mice do not show clinical signs following virus challenge, but shed virus effectively, making this a low severity model. Ultimately a natural host model such as the Welsh mountain pony must be used, but use of the mouse model enables us to reduce the number of pony experiments required.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Magnetic Resonance Studies of Cancer and Cancer Metabolism</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>MR imaging, MR spectroscopy, tumour, stroma, metabolism</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>FIVE Years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[9]</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[10]</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>It is currently impossible for MRI to ‘see’ secondary brain tumours until they are large enough to have a blood supply, so patients with lung cancer receive toxic brain radiotherapy on the basis that they might eventually develop secondaries. It is also impossible for current MRI methods to ‘see’ whether pancreas tumours are very fibrotic without taking a biopsy; those that are not would probably benefit from drugs before surgery. We are developing better imaging methods to improve future treatment for these patients. We are also investigating the metabolic effects of some cancer-related gene modifications, since there is evidence that these metabolic changes may provide a better target for new drugs than the modified genes themselves.</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>People with small cell lung cancer who do not have small brain secondaries at diagnosis and would not benefit from radiotherapy will be spared the debilitating effects of toxic brain irradiation. Patients with non-fibrotic pancreatic cancer may be allocated to successful chemotherapy before their surgery, which should improve the outcome of their treatment.
Understanding of the metabolic effects of some gene alterations in tumours will be furthered, allowing the metabolic changes to be targeted by new drugs. The work will allow further testing of a new drug designed to eliminate a tumour-related</td></tr>
</table>

[9] Delete Yes or No as appropriate.
[10] At least one additional purpose must be selected with this option.

	protein. This progress will help future cancer patients.
What species and approximate numbers of animals do you expect to use over what period of time?	We estimate the use of 1075 mice over five years .
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will use mouse models of secondary brain deposits and of pancreatic cancer, and will use a variety of MRI methods and sophisticated image analysis techniques to try to develop ways of making the small brain deposits and the tumour fibrosis visible by MRI. These mice typically receive several sequential scans. Mouse tumour models with specifically modified genes are used to measure the metabolic results of these changes by MRS. Tissues are taken at the end of the experiment for more detailed high-magnetic field MRS of the samples, and also for histological comparison. Some mice will be given drugs to see if its effects can be detected by the method used in that experiment. Mice must be anaesthetised for MRI and MRS to immobilise them, and imaging and spectroscopy in mice can be time-consuming, causing dehydration, which is readily reversible. Mice will be given well-tolerated drugs or other agents in to the abdomen or a vein, which need protection when the cannula is withdrawn. Some mice will have small blood samples taken, requiring similar care Some mice will have surgery for transplanting a tumour. They must be protected from infection, but may lose weight. Some mice may become unwell from their tumour, especially the spontaneous tumours, and must be carefully observed to pre-empt overt sickness. All animals will be killed humanely at the end of the procedures
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are developing novel techniques for MR imaging of tumours and their surrounding tissues. Tissue diversity is responsible for the enormous range of MR-detectable signals, and within that diversity, specific structures, metabolism and perfusion all contribute to small but potentially detectable differences. Tumour tissue is even more diverse than healthy tissue,

	but the detail of the determinants of many differences in signal is still controversial, and so cannot be modelled *ex vivo* . Metabolism of different tumour types is heavily influenced by specific locally variable conditions, and so cannot be fully modelled in vitro. However this project builds on much preliminary in vitro work.
2. Reduction Explain how you will assure 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.	A large proportion of the mice to be used are genetically modified to develop either pancreatic or mammary tumours. These were chosen because we are already working with these models; the pancreas model (KPC) is the mouse model that best resembles the human disease, and the mammary tumour is an excellent model for studying metabolic effects of genetic alterations. We are also use the genetically modified, but not tumour-prone siblings of the KPC mice as hosts for the same pancreas tumour with different amounts of fibrosis. Finally we will also use immune-compromised mice as hosts for human tumours grown from cell lines with metabolically relevant genetic differences. We have structured our protocols to use each mouse as its own control as far as possible, to minimise the number of animals required to achieve the objective. We are reducing the main adverse effect from MR imaging (dehydration) by limiting the scanning time, providing for regulated post-imaging rehydration strategies, and ensuring availability of expert scanner operation to ensure maximum output from time in the scanner. We are planning to use drugs and agents which are known to be well-tolerated. We have expertise in all line insertions and withdrawals in non-terminal animals. The time course and characteristics of growth of all transplanted tumours are well understood and so endpoints are rarely reached, but an efficient monitoring and reporting system operates.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Tumour Implantation for Subsequent Treatment Screening</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Tumour, Implantation</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 +</td></tr>
<tr><td>Purpose of the project (as in Article 5)[11]</td><td>
<table>
<tr><td>Basic research</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Regulatory use and routine production</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[12]</td><td>~~Yes~~</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The objective of this project is to identify drugs and treatments with the best anti-cancer properties for the specific type of tumour of each individual patient. In this way, the patient will receive one particular proven treatment regime instead of having to try several different ones in an attempt to find the best one.</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>This project will improve the patient’s potential of enjoying an extended life, an improvement in the quality of that life or even surviving the disease by decreasing the time between diagnosis and the start of a proven treatment regime.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>The mouse is the species of choice for this type of work and the number used will depend upon the success of the protocol. Previous research suggests that there is a high chance of success in which case, it is possible that around 12000 mice may be used during the 5 year duration of this project.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</td><td>Adverse effects are likely to be minimal as the tumour implantation surgery will be performed using sterile techniques similar to human surgery and the surgical technique will be very simple. Full anaesthesia will be used and the mice will receive full antibiotic and analgesic cover.</td></tr>
</table>

[11] Delete Yes or No as appropriate.
[12] At least one additional purpose must be selected with this option.

	The implanted tumour pieces are not expected to grow very large but if they extend beyond the guidelines provided by the National Cancer Research Institute, the mice will be humanely put down before they suffer any adverse effects. Between 2 to 4 weeks after the tumour implantation, the mice will be transferred to a laboratory in the USA so that they can be treated with established treatment regimes to find the best one for that specific tumour and therefore, that specific patient.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is necessary to use animals for this project as the tumours need to be in a physiologically natural environment. They must, therefore, be in living body to keep them alive until a successful treatment is determined. Also, the treatments are formulated to work in a living body and results of studies carried out in non-animal alternatives would not necessarily work in the human patient.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The current numbers anticipated are based on previous established procedures as the minimum needed to supply clinically relevant data. However, we will be investigating in-vitro techniques to try and reduce 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.	The mouse is the least sentient animal that can be used for this work. We have adopted a tumour size limitation which is more stringent than that advocated by the National Cancer research groups such that we can minimise welfare costs. We will allow the mice good time between implantation and shipping so that they can get over the implantation and be fully healthy for the flight to Baltimore. As we hopefully progress to undertaking the testing here (NPIMR) the shipping phase will no longer be necessary. The project will be reviewed 1.5 years from the start to identify any of the 3Rs which might be applicable with knowledge gained in the progress of the project.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Transgenic analysis of imprinted genes</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Imprinted genes, pregnancy, metabolism, behaviour</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)[13]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[14]</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>There is evidence to suggest that low birth weight, complication of pregnancy such as pre-eclampsia, altered metabolism in the adult (type 2 diabetes, obesity) and abnormal behaviour (ADHD, schizophrenia) all occur in response to aberrant expression of imprinted genes. Our objectives are to identify conditions with result in the aberrant expression of these genes and to use genetically altered rodents to determine the consequences of aberrant expression of specific genes on short term and life long health.</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>Identifying factors that lead to aberrant gene expression will be important in identifying preventative strategies. Determining phenotypic outcomes with respect to specific gene alterations will benefit human and animal health by identifying candidate genes for specific health complication. This information can be used to develop predictive biomarkers, identify potential treatments and also provide models in which treatments and preventative strategies can be trialled. For example, if our work resulted in a 10% reduction in the occurrence of low birth weight, 5,600 babies per year would be born in the healthy birth weight range reducing their likelihood of dying or developing ADHD, schizophrenia and/or type 2 diabetes.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Mouse.
1000 per annum</td></tr>
</table>

[13] Delete Yes or No as appropriate.
[14] At least one additional purpose must be selected with this option.

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 phenotypes we are studying are relatively mild e.g. 10-20% reduction in birth weight, mild glucose intolerance and obesity, mildly increased anxiety. The severity of our procedures is also mild e.g. intraperitioneal injection, collection of blood from a superficial vessel Consequently, we do not anticipate any adverse affects that exceed the category “mild” At the end of the protocol, animals will be culled.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Pregnancy is a mammalian-specific phenomenon which requires the physiological interaction between a female mammal, the placenta and the foetus(es) in utero. This cannot be modelled in any tissue culture system.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will apply power calculation to all our studies. We will breed our transgenes on specific strain backgrounds to reduce genetic variability. Where possible, we will breed lines as homozygotes We will cryopreserve lines we are not actively using
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 represent an excellent system in which to study pregnancy since they possess many of the same genes as humans so we can use them to study disease processes. Mice are an ideal species in which to study pregnancy due to their successful reproductive strategies and our ability to genetically modify them. We minimise suffering by applying procedures which are mild and, where possible, non-invasive.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Studies of experimental small ruminant TSE</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>TSE, scrapie, sheep</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[15]</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[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>Finish the collection of infected material from a flock of sheep naturally exposed to scrapie established under a previous project licence.
Continue the investigation of persistence of scrapie infectivity on pasture, field furniture and in buildings</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>Provision and assessment of samples of sheep of various prion protein genotypes naturally exposed to scrapie for the presence of prion protein throughout the incubation at various stages post exposure. This may aid in the development of a diagnostic test in live animals.

Inform Defra’s policy on animal health, particularly on restocking farms affected by scrapie and the need to maintain genetic resistance to scrapie in the national flock.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Up to 200 sheep over 3 to 4 years</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</td><td>The use of the recto-anal mucosa associated lymphoid tissue (RAMALT) sampling technique allows the use of a predictive end point prior to there being any adverse effects due to scrapie infection.

This technique only works in one of the genotypes</td></tr>
</table>

[15] Delete Yes or No as appropriate.
[16] At least one additional purpose must be selected with this option.

	(VRQ/VRQ). For other genotypes staff trained in the recognition clinical signs will monitor and examine the remaining sheep so they can be euthanased at the earliest point disease can be confirmed by consistent clinical signs. It is expect that this would occur when the animals show mild neurological signs which means the severity will be no more than moderate in the worst case. After euthanasia the animals will post-mortemed to confirm disease and tissues will be archived dependent on genotype and disease status of the animal.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	As yet there are no comprehensive *in vitro* alternatives to animals in scrapie research. Some aspects of the transmission barrier can be studied using *in vitro* techniques such as transfected cell lines, but these do not inform on the disease processes in the natural host, which are essential for informing Defra policies. Similarly, although protein misfolding cyclic amplification (PMCA) can detect the presence of prion protein, this *in vitro* technique is at best semiquantitative and therefore the bioavailability to the sheep cannot be predicted. Live animals exposed or infected with scrapie are required to study the presence of prion protein in samples from live animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Throughout the TSE research programme a group size of 5 sheep has been used as the minimum practical group size where possible. Although it is acknowledged that this number has statistical limitations, the questions being asked using this group size are based on the fact there is known to be a high attack rate if infectivity is present. From *in vitro*-analysis or experience if the attack rate is thought to be lower, larger numbers will be used based on statistical advice
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.	Sheep are the natural host for the disease and to answer the questions asked it is necessary to have the complete biological systems and behaviours of the sheep Use of care staff experienced in recognising early signs of scrapie infection and a welfare/endpoint action chart will prevent sheep from developing advanced signs of scrapie. In some experiments the objectives and design of the experiment allow

	sampling techniques that identify animals incubating the disease and these will be euthanased and examined post mortem before clinical signs develop.