Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 25

Project Titles and key words

- The neural basis of reinforcement learning

  Sensory processing, reward, sensitisation, basal ganglia

- Catheter based therapies for pulmonary hypertension utilising porcine models

  Pig, pulmonary hypertension, sympathectomy

- Regulation of inflammation during infectious diseases

  Pneumonia, Bacteria, Inflammation, Macrophages, Mice

- Function of the mammalian auditory system

  Hearing, Deafness, Sensory system, Development

- Targeting Heat Shock Protein 27 in colorectal cancer

  Colorectal cancer; heat shock; prognosis

- Generating and maintaining zebrafish mutants

  Zebrafish, phenotyping, mutagenesis

- Diagnostic materials for use in biological research

  Polyclonal antibodies, plant development, agriculture, cell biology

- Characterization of epithelial cell hierarchies

  Stem cells, xenotransplantation, renal grafting, mammary fat pad, cancer

- Clonogenicity in normal and neoplastic intestine

  Stem cells, cancer, epithelial biology

- The biology of normal and malignant haematopoietic cells

  Leukaemia; stem cells; haematopoiesis

- Studies of experimental small ruminant TSE

  TSE, sheep, cows, pigs

<table>
<tr><td>Project Title (max. 50 characters)</td><td>The neural basis of reinforcement learning</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Sensory processing, reward, sensitisation, basal ganglia</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></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>Yes</td><td></td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</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>Several prevalent human disorders present with abnormal sensitivities to sensory cues, including attention deficit hyperactive disorder, schizophrenia, addictions/impulsive behaviours, and aberrant salience attribution by medicated Parkinson-patients. In these cases, attention and behavioural decision-making are dominated, to a pathological extent, by sensory events. A common feature of these conditions is likely to be a malfunction in the neural systems by which associations between sensory stimuli and rewards can boost sensory processing. Understanding how such mechanisms operate normally is likely to be a necessary pre-requisite for understanding how they can be subverted by drugs and disease. Such knowledge can only help the development of rational treatments for these debilitating human conditions.

Understanding the mechanisms responsible for the modulation of sensory processing by reward will also have important theoretical consequences. Current ideas of reward-based learning focus on the effect of reward on dopamine neurotransmission in one of the brain's basic processing systems, the basal ganglia. The idea that reward can also boost processing in the sensory areas suggests that an important reassessment of reward-related learning may be required. From a practical perspective, the proposed research could also provide explanations for many related observations in the cognitive and</td></tr>
</table>

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

	behavioural sciences, e.g., the fact that our attention and gaze are particularly attracted to high value (reward-related) stimuli; how people's economic choices are often made on the basis of stimulus values; and how stimulus value can influence the speed of behavioural responses. The project will be conducted in 4 Phases with the following aims– Phase 1: as most previous experiments have been conducted with humans and monkeys as subjects we will first develop appropriate rodent models of reward-related modulation of sensory processing. Phase 2: to identify neural mechanisms responsible reward-feedback to sensory structures. Phase 3: will determine whether such conditioning can influence behavioural choice through interactions with the basal ganglia. Phase 4: to investigate how reward-boosted sensory processing influences neural plasticity and action acquisition by the basal ganglia.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This is a basic science project designed to investigate a fundamental property of brain function. There are important pathological conditions in humans associated with abnormal sensitivity to sensory cues and their ability to dominate behaviour. It is our intention to investigate the underlying neural mechanisms that enable sensory processing to be modulated by reward. An understanding of how this process operates normally may well be a prerequisite for a better understanding of how it can be subverted by drugs and disease.
What species and approximate numbers of animals do you expect to use over what period of time?	For both economic and scientific reasons the animals that will be used will be rats and mice. We will expect to use approximately 100 animals/annum on each of the two protocols. The neural mechanisms of sensory processing (vision and touch) and decision making (the basal ganglia) emerged early in vertebrate evolution and have been highly conserved throughout. These basic processes are therefore likely to be common to all mammals. Consequently, the results of this project should generalise to all mammalian species, including humans. Almost all the proposed experiments are repeated measures designs in which the responses of each subject to different experimental conditions are measure repeatedly. Therefore, statistical significance is determined within the data of single subjects. Additional animals will be required to demonstrate between

	group effects and to ensure that statistically reliable individual data are common to the group. Because we are studying robust effects our group sizes are typically small, generally less than 10 subjects.
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?	Under non-recovery anaesthesia protocols or where protocols include recovery from anaesthesia, all animals will be under carefully monitored for adequate anaesthetic depth. For protocols performed in conscious animals, additional care will be given during training and in the testing stage to ensure minimal stress and suffering to the animal. The expected level of severity for the recovery experiments is moderate. If any procedural complications do arise veterinary advice will be sought immediately from the named veterinary surgeon. In all cases the animals will be killed using an appropriate Schedule 1 method
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This is a systems-level neuroscience project that will investigate a fundamental aspect of brain function. At present there is no alternative to using invasive experiments with animals to gain the knowledge required. However, we have 15 years' experience of working in close collaboration with computational neuroscientists who use our biological data to parameterise their computational models of basal ganglia function. The progressive refinement of these models will gradually replace the need for biological testing.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Repeated measures designs are typically associated with the intensive study of comparatively few subjects. For this reason most of the research proposed in this projects entails repeated measures designs. An additional factor reducing the numbers of subjects required is the simultaneous acquisition of multiple measures of neural and behavioural activity.
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.	Sensory interactions with the rodent basal ganglia is the model of choice for the proposed experiments. We have over 30 years' experience working with rodent sensory systems and for understanding basal ganglia functional architecture. Both systems are highly conserved in the vertebrate brain so our results will generalise across species, including humans.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Catheter based therapies for pulmonary hypertension utilising porcine models</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Pig, pulmonary hypertension, sympathectomy</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>3</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[3]</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[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>Pulmonary hypertension, high blood pressure in the lungs, is a devastating disease that causes death and significant suffering among patients. The drugs currently used to treat this disease cost up to £300,000 per patient, per year and are only minimally effective. Over active nerves supplying the blood vessels of the lungs contribute to the high blood pressure. Surgical resection of these nerves significantly reduces this pressure; however, open chest surgery carries high risk in patients. A new catheter-based technique for cauterising the nerves surrounding the lung blood vessels using radio-frequency has recently become available. This procedure may make it possible to remove the nerve supply to the arteries of the lungs, as achieved by surgery, using a minimally invasive technique that are very similar to that required for diagnosis. We have recently generated proof-of-concept data in a non-recoverable acute pig model and now aim to determine the long-term effects to both the vessel wall, and pressure. Furthermore, we aim to determine the optimal and minimal number, and location of radio-frequency treatments. Finally we will establish and test this new therapy in a pig model where pulmonary hypertension is already established.</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>If successful, this procedure could quickly result in a human clinical trial with the potential for a new treatment for a devastating disease with no current cure.</td></tr>
</table>

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

What species and approximate numbers of animals do you expect to use over what period of time?	Pigs, approximately 60 over 3 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 animal will be under general anaesthetic throughout the catheter procedures, so discomfort to the animal will be minimal. The injection of sedative and induction of general anaesthetic may cause discomfort. There is a very small risk of death from the general anaesthetic. The insertion of tubes into the blood vessels may cause bleeding, which will be treated appropriately at the time. The radio-frequency catheter passed into the blood vessels may cause damage to, and bleeding from the blood vessels. The animals recovered from anaesthesia following the radio-frequency energy delivery will be carefully monitored to ensure they are well enough to eat and drink. Pigs that are induced to develop pulmonary hypertension will be carefully monitored during the disease time-course and will be sacrificed if they display evidence of severe disease. Evidence for any side effects will be carefully monitored for during the procedure. All animals will be killed under anaesthesia at the end of the procedure. All recovery procedures are rated as moderate severity.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The complex physiology of the heart and lungs cannot be replicated in non-animal alternative. Pre-clinical studies are required to test determine efficacy prior to human trials.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have carefully selected the models to minimise animal numbers. For example, we can perform a radio-frequency treatment and then immediately test the effect on blood pressure.
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 pig is physically, biologically and genetically similar to man. We make every effort to reduce suffering particularly through the use of general anaesthesia. In the recovery models the pigs will be closely monitored to minimize harm.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Regulation of inflammation during infectious diseases</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Pneumonia, Bacteria, Inflammation, Macrophages, Mice</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)[5]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Regulatory use and routine production</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Preservation of species</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Higher education or training</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[6]</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>We will define elements of the body’s immune response that maximizes bacterial clearance in the lung and minimize the potential for harmful effects of the inflammatory response, which may damage the lung, using mouse models of lung infection. This knowledge will be used to identify ways in which we can retune the body’s response to pneumonia to improve patient outcomes testing these in our mouse models.
Our approach to treating pneumonia relies exclusively on antibiotics and is challenged by increasing antibiotic resistance. Healthy people are intermittently exposed to bacteria that cause lung disease but their body’s defences prevent pneumonia. The reasons why a small subset of people are susceptible to pneumonia is unknown. We will identify key aspects of the protective response against lung bacteria using models we developed in which most mice clear bacteria. We have defined factors that regulate bacterial killing in immune cells in the lung and also mechanisms regulating inflammation. We will focus on these findings to identify the essential factors controlling the optimal response to bacteria in the lung and how we can manipulate these factors to improve pneumonia outcomes.</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 investigations in mice when combined with our use of human cells and patient samples will help us define the key parts of the body’s response to lung bacteria that prevents pneumonia or reduces its severity. In addition it will suggest how</td></tr>
</table>

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

project)?	we can improve this response to prevent or reduce the severity of pneumonia in patients or animal populations at risk.
What species and approximate numbers of animals do you expect to use over what period of time?	We study mice because their immune system and lung anatomy is similar to man. There are many resources available for use with mice and genetically modified mice are available to test key factors controlling the immune response. We test key hypotheses in human cells or in patient samples *in vitro* before studying mice to reduce numbers. We collect the maximum possible number of samples to reduce mouse usage. We refine our methods to ensure reproducibility and calculate group sizes carefully using validated statistical methods with the input of a statistician. We estimate we will use approximately 40700 mice in the lifetime of this license.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Mice will most often receive bacteria directly into the lung which requires a short surgical procedure under anaesthesia to identify and put a small cannulae into the windpipe (intratracheal instillation). Mice recover rapidly and there is only minimal suffering. This model is used as it most closely mimics the aspiration of bacteria by humans. For many of our studies we use low doses of bacteria and infection is sub-clinical. When we do establish pneumonia we try and ensure that the disease is not too severe. All mice are carefully monitored for signs of ill health and are culled before they show any signs of distress. When we establish infection at a different site, to compare the response in the lung with other sites, we will also monitor closely for any signs of ill health.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We require mice because we cannot study complex multicellular interactions in cell cultures. Experiments using multicellular human tissues are not yet refined enough for our studies while important differences between invertebrate and vertebrate organisms mean we cannot replace mice with invertebrates, although we will continue to explore their potential use.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will refine models as outlined above to reduce assay variability thus reducing group sizes. We will continue to explore the potential of small animal imaging to allow collection of data at multiple time points and will maximize and refine the collection of multiple pieces of data from individual mice. Enhancing reproducibility and collecting multiple data points from individual mice also reduces

	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 investigation of the early stages of infection and inflammation ensure minimal suffering. The intratracheal instillation of bacteria involves a short surgical procedure without ill effects. Breeding mice with genetic modifications to study the working of the immune system does not involve the creation of major congenital defects or clinical illness and mice do not have features of ill health. If the mutation makes them susceptible to naturally occurring infections these are prevented by filtering air and the use of antibiotics. Similarly although bone-marrow transplantation involves conditioning with radiotherapy the treatment is broken down into low doses to minimize adverse effects and natural infections are prevented with filtered air. Modifying diet to a western style diet does not cause ill health. The wound-healing model is the least invasive way of studying tissue repair under conditions of altered inflammation and with minimal distress and rapid mouse recovery.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Function of the mammalian auditory system</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Hearing, Deafness, Sensory system, Development</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)[7]</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>
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<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[8]</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>How biological systems orchestrate their development and how complex signals are processed by mature neuronal networks are major challenges in the quest to understand human biology and disease.

The objectives of the proposed research is to define the critical physiological steps and underlying molecular mechanisms required for the maturation and function of the mammalian sensory hair cells, in the auditory and vestibular systems, and their fibre connections from/to the brain.</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>Hearing is one of the key senses that allow humans and other vertebrates to acquire important information from the surrounding environment. When mammalian cochlear hair cells are damaged, they cannot be replaced and this is the reason why hearing loss is an irreversible process. Hearing loss is a very common disorder affecting 360 million people worldwide (WHO 2013: http://www.who.int/mediacentre/news/notes/2013/hearing_loss_20130227/en/).

This project, by determining the main elements required for hair cell function and development we will be able to support translational/clinical research, which is directly aimed at developing a cures for hearing loss, as well as progress the technical development of hearing aids, including cochlear implants. The scientific advance generated by the proposed study thus offers a great social benefit since it would contribute, in the long term,</td></tr>
</table>

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

	to decreasing the number of people with permanent hearing loss.
What species and approximate numbers of animals do you expect to use over what period of time?	The work will be performed on transgenic or mutant mice. We will used a few thousands animals over the duration of the proposed research (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 current proposal only uses non-invasive procedures. The severity will range from Mild to Moderate, which mainly related to the fact that some of the mice have harmful mutations. Animals will be culled at the end of procedures using approved methods. While the proposed procedures will themselves not cause recognized pain/suffering/distress/lasting harm to animals, they will be regularly monitored for their ability to eat, drink, ambulate, socialize/behave, gain and generally thrive well compared to wild type equivalents. Any animal showing any cause for concern by experienced staff will be carefully monitored and, where necessary, additional advice from the NACWO or NVS will be sought.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Unfortunately, there is no substitute for the integrated sensory systems or functioning brain in a living animal in order to understand how they work together.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals used will be kept to a minimum by making sure that all experiments are meticulously designed and by ensuring a high level of training for the staff involved in the various procedures. We also use a rigorous statistical approach to all our 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.	1) The use of the rodents as a model demonstrates the use of a species of a low neurological sensitivity. 2) Moreover, mice are an excellent model system for hearing in mammals because there is a great deal of similarity in the two auditory systems at the anatomical, molecular and physiological levels. 3) Personnel are thoroughly trained and carefully supervised until they achieve high competence in the regulated techniques they will apply.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Targeting Heat Shock Protein 27 in colorectal cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Colorectal cancer; heat shock; prognosis</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>Two</td></tr>
<tr><td>Purpose of the project (as in Article 5)[9]</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[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>Our main objective is to identify whether inhibition of heat-shock protein 27 (HSP27) in colorectal cancer can improve response to chemotherapy. HSP27 is produced by normal cells in response to stress and it protects them from undergoing cell death. Our previous studies found that colorectal cancers can have abnormally high levels of HSP27; this predicts poor prognosis and correlates with worse response to chemotherapy. It is not known whether blocking production of HSP27 in colon cancer cells can improve chemotherapy sensitivity. We plan to generate colon tumours in mice and test a new drug inhibitor of HSP27 alongside conventional chemotherapy. Importantly, tumours will be seeded directly into the colon of mice and growth and treatment response monitored using a state-of-the-art animal scanner. We rejected using the historical technique of implanting cells into the flank as this produces tumours with altered growth and chemotherapy response profiles.
Our second objective is to quantify changes in the tumour-associated immune response after blocking HSP27. White blood cells (WBCs) migrate out of circulation into tumours deposits and can attack the cells, reducing the chance of metastasis. However some tumours produce factors which damp-down this response, inducing WBCs to assist cancer metastasis. HSP27 is one of these factors. The host mice and implanted mouse cancer cell line in our study have the same genetic background. This ensures that the implanted tumour will generate a typical immune response without rejection; allowing us to study the effect of HSP27-blockers on tumour-associated WBCs.</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>A HSP27-blocking drug is currently being tested in humans with bladder and prostate cancer following studies in mice. Colorectal cancer is the second most common UK cancer and novel drugs which increase operability and improve prognosis are sorely needed. This study will provide data on whether HSP27-blockers are worth pursuing in clinical trials.</td></tr>
</table>

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

What species and approximate numbers of animals do you expect to use over what period of time?	Fewer than 130 mice over 24 months.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The project is in two phases. In the first experiment the mouse-derived cancer cells will be genetically-modified to create two sub-types; these will be identical except that Type A will produce HSP27 (HSP27+) and Type B will not (HSP27-). A small number of mice will be randomised to receive either cell-type A (HSP27+) or cell-type B (HSP27-) implanted in the colon under general anaesthetic. The growth and spread of the tumour will be measured with a weekly scan under anaesthetic. All mice will be humanely euthanized at 42-49 days post-surgery allowing post-mortem tumour measurements and blood samples to be taken. In the second experiment, a second cohort of mice will have tumour cells implanted in the colon. They will then be randomised to receive one of three treatments; placebo, standard chemotherapy or chemotherapy plus HSP27-blockers. The response to treatment will be monitored using weekly scans and mice will be euthanized at 42-49 days for post mortem examination. Mice are expected to make a full recovery from abdominal surgery under general anaesthetic, facilitated by administration of analgesics and antibiotics. The implanted tumours are not anticipated to cause any immediate symptoms and the severity of the protocol is expected to be moderate. Chemotherapy treatment may uncommonly cause gastrointestinal upset and increased susceptibility to infection. The HSP27-blocking drug has no recorded side-effects in mice. Weight loss is the most common complication of tumour growth and all animals will be weighed and have their condition assessed daily.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are already studying HSP27-blockers in Primary Cell Culture (PCC). PCC is a technique which grows cancer cells directly from a human tumour; it better reflects the diversity of cancer cells within a tumour than cell lines. This should reliably predict the magnitude of the effect of HSP27-blockers in solid tumours. However no laboratory models are able to reproduce or predict the interaction between cancer cells and WBCs seen in real tumours. It is therefore vital for us to use an animal model to establish the effects of HSP27-blockers on both chemotherapy response and the tumour-associated WBCs.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We designed our experiments in two phases as statistical data on tumour growth and variability in this model is not available in the literature. Data generated in the first phase of the project will be analysed by Professor Preziosi prior to commencing the second phase so that the minimum number of mice can be used in each treatment group. Our choice of model utilises the least number of animals possible.

	Similar results could be obtained by inducing colon tumours in genetically-modified HSP27-positive mice. This would require the sacrifice of a large number of breeding animals. Also, inducing spontaneous colon tumours using drugs in mice is imprecise and causes GI side-effects. More mice would be needed in each group to ensure an adequate number of tumours for study. The use of cancer cell implantation in immune-competent mice meets the objectives of our study and is likely to require fewer than 130 mice.
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 animal model chosen is the most efficient to achieve our objectives. By modifying the cancer cells in the laboratory prior to implantation we gain precise control over HSP27-expression in the tumour. Tumour growth is more predictable following cell implantation than spontaneous-induction with drug treatment. Implantation in the colon rather than the flank will ensure a reliable response to chemotherapy; and high-tech scanning ensures that tumour growth can be measured without sacrificing animals. Our immune-competent mice are less prone to infection than commonly-used nude mice which should ensure rapid recovery following surgery. Implantation of tumour cells will be performed under anaesthetic by trained surgeons. This should ensure a swift, skilful procedure with minimal blood loss or tissue trauma. Analgesia and antibiotics will be administered to ensure a pain-free recovery with minimal complications. Imaging will be performed under anaesthetic to minimise stress. Wherever possible blood will be taken only under terminal anaesthesia. It will not be possible to prevent some animals experiencing weight loss or lethargy related to tumour growth; however weight and general condition will be monitored daily and any animal in distress will be humanely euthanized.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Generating and maintaining zebrafish mutants.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Zebrafish, phenotyping, mutagenesis</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)[11]</td><td>
<table>
<tr><td>Basic research</td><td>Yesx</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>
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<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[12]</td><td>YesX</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>This project aims to create a genome-wide resource of zebrafish mutants for research that will be used to develop human disease models and characterise the functions of a selected set of genes.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The best way to study the function of a gene in a complex embryo or animal to disrupt its function and study the consequences. Recent advances in genome technology have made it feasible to create a mutant line for every gene in the zebrafish genome. These mutants can be maintained as frozen sperm samples and accessed when needed. Such a mutant collection will be immensely useful as a source of fish that exhibit phenotypes similar to human diseases (disease models) or which can be used to test the functions of other genes in normal development and physiology.</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 maximally 5000 zebrafish (Danio rerio) per year over 5 years.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</td><td>In most cases we will work on embryos, therefore adult fish are only required in natural matings to generate these embryos. Occasionally, to identify the correct genetic type of animal we will take a small part of the fin, which will regenerate and does not significantly impair fish well being. In some cases, fish may be anaesthetised if they are required for e.g. fin clips, observation, or expression of oocytes. Anaesthesia may cause light transient bleeding from the gills. Rarely, fish may have</td></tr>
</table>

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

	difficulty waking up or very rarely they may fail to recover at all. Over the 5 years of the project we may treat up to 100 fish with mutagenic chemicals that damage DNA, such as ENU. This mutagenesis work is a central part of the project because it aims to create animals with defective genes and is unavoidable. This treatment can cause stress and weight loss to the fish for a limited amount of time, and over the longer term, the treated fish may develop tumours. Such animals will be monitored carefully and they will be euthanized if their well-being is significantly affected.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our aim is to create a large resource of which can be used to study every gene in the zebrafish. The resulting mutants will be used to create animal models of human diseases or to understand the role these genes play in development. This can only be done properly in the context of an animal embryo. Treatment of sperm with DNA damaging agents cannot be done in vitro, as such treatment would not be expected to result in normal embryos> Germline stem cell cultures might be useable, but they are not yet available in fish.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Over five years, we estimate that we will maximally use 5000 adult zebrafish per year. These fish will generally be only used for matings to create embryos for study or to maintain lines. These numbers are determined by stock-keeping requirements which dictate that a minimal number of adults are required per mutant line to ensure availability. Where live stocks of mutant lines are not continuously required, they will be stored as a frozen as sperm sample. 100 adult fish will be required for the treatment with DNA-damaging mutagen. This number is based on the fact that we need to produce 4000 male progeny to establish our mutant resource and we can only raise a limited number of fish per mutagen-treated 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.	We are using the fish to create genome-wide resource of mutants to study animal development and disease. Defects in gene function often have consequences that are not direct but are a result of interplay between different tissues. Similarly, diseases are often the end result of multiple interacting processes, rather than based on a single form of cell dysfunction. Such conditions are difficult to recreate using cell culture systems. Our choice of animal also reflects the 3Rs, since fish are the simplest model vertebrate in which these studies can be performed, and our experiments are on embryos or larvae which have a lower level of neurophysiological sensitivity relative

	to adults. Most of our experiments will be done with embryos less than 5 days old, and thus will fall outside the Animals (Scientific Procedures) Act.

Diagnostic materials for use in biological research
Polyclonal antibodies, plant development, agriculture, cell biology

- Summarise your project (1-2 sentences)
  This project is aimed at production of antibodies against novel antigens (proteins, glycoproteins, polysaccharides) where no adequate commercial source is available.

- 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.
  As the primary food source, understanding the biology of the plants is essential for the future benefit to mankind. The increasing pressures on agriculture to provide high quality food and non-food crops more efficiently and in an environmentally sympathetic manner demands intensive research of the function of the structural blocks of plant body - plant cells. In particular such studies include analysis of the chemical reactions which determine how plants grow, how they respond to different environmental conditions, how plant cells and organ systems differentiate and how these processes can be manipulated to produce future 'designer' crops with higher yields or nutritional composition by conventional plant breeding or by genetic modification. Some processes that occur inside plant cells are conserved in the cells of other living organisms including animals and microbes and it is important to have thorough understanding of these processes for the efficient crop design. One of the most commonly used tools for studying biological properties of the molecules that compose cells is antibody. Antibody can reveal localisation, concentration, modifications of a molecule as well as identify its interaction with other molecules during development, stress etc.

- Outline the general project plan.
  This program aims at production of antibodies against biological molecules in mice, rats and rabbits. The procedure starts with the design, synthesis and purification of the molecules of interest. They will be afterwards injected into the animal like a routine jab is done. The injection will be repeated to increase concentration of antibodies in the blood. At the peak of the concentration, the antibodies will be purified from blood.

- 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 making the antibody a molecule to be studied, will be dissolved in water-based solution and mixed with harmless medicines that will lead to the enhancement of the immune response. This mixture will be injected under the skin or into the muscle like a usual immunisation. When the concentration of the antibodies in the blood stream reaches the highest point, animals will be given anaesthetic and their blood will be collected for preparation of the antibodies. Considering the harmless nature of substances used in this project, no adverse effects on the animal is expected.

- Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.
  This project will generate novel tools to study molecules in plants leading to better understanding of plant biology and facilitate invention of new strategies for improving crops.

- 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.

  The project will use laboratory-bred mice, rats and rabbits. No more then 102 animals will be used for the whole duration of the project. All these animals have been chosen because of very well studied immune response, a wealth of information about immunization techniques and easy of maintenance. The whole antibody production strategy has been designed so that the minimum number of animals will be used in order to obtain antisera of sufficiently high quality. The design considers the specificity of the immune response of each animal and qualities of the targets (antigens) used in each experiment.

- 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.

  Antibodies are produced in the laboratory animals. There are no convenient alternatives for the production of antibodies. A known procedure of production of antibodies in bacterial cells still requires the use of laboratory animals in order to obtain the gene that encodes this antibody from spleens of the animal. This technique is too labour intensive, prone to errors, time consuming and costly.

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

  This program does not use any harmful substances. It based on the ability of immune system to recognise foreign molecular in the body and neutralise them using immunoglobulins. The experimental animals used in the programme will be subject to the high quality care and daily veterinary inspection and experiment will be stopped in case of any adverse effects will become noticeable.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Characterization of epithelial cell hierarchies</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Stem cells, xenotransplantation, renal grafting, mammary fat pad, cancer</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[13]</td><td>
<table>
<tr><td>Basic research</td><td><u>Yes</u></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td><u>Yes</u></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[14]</td><td><u>Yes</u></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 main focus of this project proposal is to identify and characterise the different types of cells that make up the normal mammary and prostate glands in both human and in mice, and in human breast, prostate and ovarian tumours. We are particularly interested in identifying which cells function as the stem (mother) cells, and determining what types of daughter cells they generate. Understanding these parent-progeny relationships are very insightful because evidence has emerged that these relationships are often maintained in cancer, and may explain the behaviour of tumours and the emergence of therapy resistance. For example, the more mature daughter cells may be sensitive to therapy, and killing of these cells can shrink the tumour, but if the more primitive mother cells (e.g., the cancer stem cells) are resistant to the therapy, then they will just proliferate and cause the tumour to regrow. Identification of the molecular pathways of these therapy-resistant mother cells would help in the design of a treatment that could complement existing therapies. A minor focus of this project proposal is the identification of the molecular mechanisms that regulate the differentiation of mammary stem cells into the muscle-like cells that squeeze milk out of the mammary gland. We are interested in these mechanisms since they could be exploited to turn cancer stem cells into a non-growing state.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be</td><td>Identification of molecular pathways that drive cancer growth. These pathways, once identified, could be targeted using exiting or yet to be</td></tr>
</table>

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

advanced or humans or animals could benefit from the project)?	developed drugs.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use approximately 5,400 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?	Most of the animals that we propose to use will be used as hosts for growing normal and malignant cells. We use surgical techniques to implant normal cells, either to the kidney capsule or into the mammary gland. Our experience has shown that ~95% of mice will recover from these surgeries with no adverse effects. Problems, when they do occur, are usually associated with wound closure after surgery or when female mice are injured by male mice in subsequent mating experiments. When we implant tumour cells, we usually do this as an injection under the skin. The main problem associated with tumour growth in this body site is the ulceration and scabbing of the skin above the tumour. Some of the mice are expected to show some ulceration in the skin during tumour growth, although in most of these cases, the skin will heal within a few days. In some cases, the ulceration will progress, and these mice will have to monitored closely to prevent this exceeding a moderate degree of severity. Some of the mice will be used to maintain breeding colonies. The vast majority of the animals used for this will not experience any adverse effects. All animals at the end of the experiments will be killed in a humane fashion.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In vitro methods that promote the growth of normal and malignant breast, ovarian and prostate cells and maintains them in a state that mirrors the *in vivo* state have not been developed. As a result, transplantation of cells into mice still remains the only method of growing and maintaining tissue in its natural state.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We reduce the number of mice used by a variety of methods: 1. Using appropriate experimental design (e.g., transplanting appropriate numbers of cells such that engraftments give useful information, and performing statistical tests in advance in order to identify the minimal number of mice required for the experiment)

	2. Transplanting multiple grafts per mouse. Transplanting multiple grafts in a mouse often does not increase the severity of the adverse effects that mouse will experience, but it can drastically reduce the number of mice required. 3. Performing cell culture experiments beforehand in order to see if an experiment is worth continuing in an animal model.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Only specific genetically modified mice can be used for growing human tumours since only these animals are immune-deficient enough to permit tumour growth without immune-rejection. We have spent considerable time in the past working with the veterinary surgeon to optimise our surgical techniques. The vast majority of our mice recover from surgery without any complications. As discussed above, we have selected the least invasive method (injection underneath the skin) for growing tumours in mice. We prefer this site because it is easy to monitor tumour growth, it is not an invasive procedure and there is less risk of compromising the function of internal organs. We prefer implanting our normal cells under the kidney capsule because it is easy access, the mice recover well from this surgery and we can implant up to 6 grafts per mice using this method, which minimizes the number of mice required. In all experiments, we practice good surgical techniques and we work with the animal facility staff (and when required, the veterinary surgeon) to closely monitor mice that have had surgery or have undergone other regulated procedures. Mice that display a level of adverse effects that reach a pre-defined level are killed in a humane fashion.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Clonogenicity in normal and neoplastic intestine</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Stem cells, cancer, epithelial biology</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>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>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>We want to understand how the molecular and genetic events that drive cancer change the cellular biology of colorectal cancers, particularly with respect to the stem cells from which all the cells ultimately arise.

Hundreds of mutations are being found in different cancer types including colorectal cancers. The significance of this is still to be established but obviously it will take considerable time. Despite many different inputs there are only a finite number of ways cells can respond and we are trying to find new ways to describing how stem cell behaviour is regulated normally and changed in cancer.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The combination of reducing extensive molecular heterogeneity to a finite number of cellular behaviours and, moreover, evaluating the functional consequence of such behaviours for tumour growth we hope to identify either new cellular properties for therapy and also to identify how existing treatments may be modified to target cellular subsets. More fundamentally we will understand how stem cells behave and are regulated so that they can maintain our tissues normally and during injury and disease and thereby offer new approaches to regenerative medicine and inflammatory disease.</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 up to 40,000 mice over five years.</td></tr>
</table>

[15] Delete Yes or No as appropriate.
[16] 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?	Our plan is to use, refine and develop mouse models to study the cellular biology of intestinal cancers throughout the different stages of cancer, namely initiation, progression and metastasis. Having identified the subsets of cells that maintain cancers we will test the efficiency of interventions that preferentially target them in causing tumours to regress. Mice need to be bred that will develop cancer either spontaneously or induced by treatments. Some of these animals need to be maintained and treated while we attempt to identify, understand and modify the nature of the cells that maintain their cancers and to determine the consequences of doing so.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Genetic modifications will only be introduced into live mice where preliminary in vitro study confirms that they are functional. In vitro approaches will characterise how manipulations of molecular pathways modify some aspects of cell behaviour. However, study of tumour cell behaviours requires the interaction of many different cell populations and dynamic interactions that can only be found in a living animals and that act to maintain cancerous growth over many months.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal numbers are minimised by designing breeding strategies to produce experimental mice as efficiently as possible. Animals bred that lack some of the genes for experimental study are often used as controls. Statistical advice ensures that appropriate numbers of animals are recruited to individual studies. Preliminary experiments using small numbers of mice address feasibility before undertaking larger 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.	Mice will be used because they can be easily genetically manipulated and develop tumours over a reasonable timeframe that permits their study. Most animals, for most of their lives will not experience suffering. All animals will be monitored for signs of distress and pain and interventions such as analgesia given if needed. Humane endpoints will be put in place and continuously re-evaluated and animals killed if necessary.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>The biology of normal and malignant haematopoietic cells</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Leukaemia; stem cells; haematopoiesis</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)[17]</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[18]</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>The purpose of this project is to identify stem cells in normal and leukaemic bone marrow samples and to validate the role of particular candidate genes and cellular pathways in their function. [32]</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The expected benefits of this project include new knowledge regarding the requirement for several genes or cellular pathways for the function of normal bone marrow or leukemia stem cells; identification of new candidate therapeutic targets for the treatment of leukaemia; and evaluation of the efficacy of novel candidate therapies in pre-clinical studies. [52]</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>Most procedures in the protocol, such as irradiation of animals, blood or bone marrow sampling, or injection of cells and compounds are not associated with significant side effects and are classed as of moderate severity. Mice injected with leukaemia cells will, when the disease develops, exhibit signs of disease, such as hunched posture, piloerection and poor levels of socialising and interaction. Under these circumstances, and whenever else an animal displays features of ill health, or at the end of each experiment, mice will be humanely euthanized using a Home Office sanctioned method. [92]</td></tr>
<tr><td>Application of the 3Rs</td><td></td></tr>
<tr><td>1. Replacement
State why you need to use animals and why you cannot use non-animal alternatives</td><td>Stem cells are defined by their ability to regenerate normal or leukaemic bone marrow cells following their transfer in to a second animal. In vitro experimental systems are insufficient for this purpose because they do not provide the required cellular and host environment for the development</td></tr>
</table>

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

	of a new tumour or normal bone marrow system This is because these entities typically have very complex cellular architecture, involving interactions between many different cell types which cannot be reproduced *in vitro*. Thus without the use of a live, whole animal experimental system, the biology of bone marrow stem cells cannot be meaningfully studied. Use of animal models of leukaemia is essential for a second reason. One of the drawbacks of experiments using human leukaemia cells is their genetic variability making the accurate and meaningful study of the effects of specific genetic lesions in isolation very difficult. By contrast, murine models of human leukaemia enable investigation of the biological effects of specific genetic lesions in a tractable, controlled and highly informative manner. [169]
2. Reduction Explain how you will assure the use of minimum numbers of animals	Use of animals will be minimised by (i) making use of *in vitro* model systems wherever scientifically justified, (ii) use of *in vivo* bioimaging or bone marrow aspiration to follow disease development and response in real time (rather than culling cohorts of mice at defined time points), (iii) careful experimental design informed by the expert advice of a statistician (consulted regularly) so that the minimum number of mice required to produce a scientifically acceptable result are used, (iv) the use of pilot experiments to test for the extent of an expected phenotype prior to a full scale confirmatory experiment (thus avoiding full scale experiments that may lack sufficient statistical power), (v) the use of protocols for each experiment which include the objective, proposed interventions, numbers of animals and analysis method (reviewed in every case by the licence holder and BRU staff for experimental rigour and the 3Rs) and (vi) the cryopreservation in multiple aliquots of leukaemia samples (which eliminates a requirement for continuous production of cohorts of mice with experimentally initiated AML). [172]
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice have been chosen for the study because they represent the least sentient species from which meaningful experimental data can be generated, while exhibiting considerable genetic and biological similarities to humans with regard to their blood forming system. Only a mammalian haematopoietic model system has the potential to accurately mimic both the anatomy and complex cell biology, including micro-environmental interactions, of human normal and leukaemic haematopoiesis. Furthermore, there is considerable experience in

	the wider scientific community regarding the use of mice as a model system for human haematological malignancies and many reagents exist for the phenotypic characterisation of mouse cells. The techniques used have been carefully evaluated to minimise distress to the animals. Mice used in surgical procedures will be treated with anaesthesia, analgesia and post-operative rehydration by subcutaneous injection, followed by careful observation. In other areas, irradiation doses will be administered at a level sufficient to induce bone marrow suppression but no other long term sequelae; bone marrow injections and aspirates will be not be performed routinely, only where the scientific justification is high; the risk of rejection of neonates by their mothers following transplantation procedures will be minimised by limiting separation times; and in studies that result in the initiation of leukaemia, animals will be closely monitored for health status and killed by a Home Office approved method when signs of ill health are displayed. [227]

<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, sheep, cows, pigs</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)[19]</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[20]</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>Naturally-occurring TSE arise as a number of distinct, identifiable ‘strains’ (eg classical and atypical BSE in cattle, classical and atypical scrapie in sheep). If these strains were to cross from one host species to another, either naturally or through animal feed, we need to know what these would look like, and whether or not our current surveillance sampling and testing methods are robust enough to detect and classify them.</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>Knowing which species are susceptible or resistant to specific infections helps the policy makers and risk managers decide which disease controls and food production measures are important for protecting the animal population and people. Knowing as much as possible about what a disease looks like increases the chance of us being able to detect it effectively if it should occur, further strengthening animal health and human food chain protection.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>The project will use 107 sheep, 12 cows and 4 pigs.</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 animals have already been challenged with various TSE, and are now being housed under normal conditions until they show signs of disease. They will be monitored daily by the animal care staff, and have regular more detailed clinical examinations. When any abnormality, such as a change in behaviour, movement or eating habits, is noted the monitoring increases, so that any clinical</td></tr>
</table>

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

	signs can be properly monitored and described. When the animal is showing definite signs of disease it will be killed humanely, and a full range of samples taken for a range of tests to characterise the disease type.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	If you want to know what the clinical disease will look like in a particular species, or whether that species might be resistant, you must use that particular species.
2. Reduction Explain how you will assure the use of minimum numbers of animals	A study which uses farm animal species is necessarily limited in scale. These are pilot studies which aim to establish resistance or susceptibility to a particular agent, or by a particular route of challenge (eg can lambs contract the disease from consuming milk) before any quantitative study is planned. In this way, larger studies to look at, for example, minimum infective dose, would only be started once susceptibility has been established.
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.	As indicated in section 1, if you wish to describe and characterise a disease in a given species, then you have to use that species as your model. There are several transgenic mouse models that are commonly used in TSE research for the characterisation of disease isolates, but if you need to know the clinical signs or tissue distribution of the pathogen in a particular species you cannot extrapolate from another species. All animals will be closely monitored for any clinical or behavioural abnormality, and such changes carefully recorded. If necessary, CCTV will be used to look for infrequent or subtle signs which may not be displayed when observers are physically present. We have many years of experience with a range of TSE in a range of host species, and we have established clearly defined, disease-specific endpoints. This list is continually reviewed in light of data emerging from other TSE challenge studies, and as published in the scientific literature. The decision-point for culling an animal is when any abnormality is deemed to adversely affect the animal's normal activity.