Home Office

# Animals (Scientific Procedures) Act 1986

## Non-technical summaries granted during 2013

## Volume 11

## Project Titles and key words

- New mouse models of human disease

  ENU, Mutagenesis, phenotyping, Age-related

- Interventions against respiratory pathogens

- Effect of incubation on post-hatch development

  Avian, incubation, muscle, myofibre, thermal

- Immunological control of autoimmune disease

  Type 1 diabetes, therapy, immune system

- Defining and exploiting glycosylation in trypanosomes

  Trypanosome; glycosylation; drug targets; diagnostics

- Genetically altered rodent production in support of research programmes

  Production, genetically altered, human disease

- Vaccination against TB in murine models

  Bovine Tuberculosis, mice, vaccination, protection, Biomarkers

- Antibodies for research and assays

  Antibody, Monoclonal, Neuromuscular, Diagnosis, Monitoring

- Mouse models of cancer progression and therapy

  Cancer, melanoma, oncogene, metastasis, therapy

- Mechanisms of normal and malignant haematopoiesis

  Blood cells, leukaemia, chemotherapy, cancer genes

<table>
<tr><td>Project Title (max. 50 characters)</td><td>New mouse models of human disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>ENU, Mutagenesis, phenotyping, Age-related</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)[1]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Translational and applied research</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Preservation of species</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Higher education or training</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</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>
<ul>
<li>To assign functions to and identify novel functions of genes</li>
<li>To identify genes and pathways associated with disease</li>
<li>To create new models of human disease</li>
</ul>
Through this project we aim to gain a better understanding of the pathways that can lead to disease through the manipulation of the genome and in some cases combined with environmental challenges such as ageing. We will also investigate the modification characteristics to understand more complex diseases. Despite great advances in our understanding of human disease there are limitations to the study of patient groups in associated individual genetic changes to disease phenotypes, primarily because of the outbred nature of human genetics and the complex environmental differences experienced by patients. We have a particular interest in age-related disease, an area where our understanding of the genetic contribution to disease is lacking.
</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 provide data on the functions specific genes for many disciplines of biological science. By analysing this information it will be possible to ascribe function to some of the genes in the mouse genome and to extrapolate such information to humans showing similar disorders. By identifying genes, and variations in genes, that result in or contribute to disease we will improve our understanding of how and why diseases develop and also potentially identify new targets for</td></tr>
</table>

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

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| | therapies. |
| What species and approximate numbers of animals do you expect to use over what period of time? | We will employ mice, up to 65,000, over 5 years. This is a large scale collaboration involving many scientific groups. |
| 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 generated that carry a range of genetic changes, similar to the variation seen between individual human subjects, and the effect of these variations analysed. We will employ a range of tests, many of them very close to those patients undergo in hospital, to identify mice with characteristics of disease. The mice will be aged to around 18 months of age, undergoing a variety of investigations throughout their life and then will be culled humanely. We are investigating disease and hence mice will develop symptoms seen in patients. Because of the comprehensive nature of our testing we will expect to detect disease in its early stages and thus prevent undue suffering. |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | Model organisms are vital in determining the function of all genes as their genomes can be manipulated using genetic engineering. Intact organisms are important as the action of many genes is far ranging, affecting different cell types in different organs of the body. The mouse occupies a unique position as a model for genetic research as it demonstrates remarkably similar development, physiology and biochemistry to the human. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | The programme involves a large number of scientific groups and a comprehensive examination of each mouse. By sharing the mice we will therefore extract the maximum amount of information from each unique mouse and have the capability to carry out further investigations. The number of mice being tested is determined statistically and the number of mice produced matched to the capabilities of the research groups. |
| **3. Refinement**<br>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. | Most of the tests employed in this research programme are non-invasive and cause little or no distress to the mice. We wish to investigate the cause of disease, something difficult to do in patients, and will not allow disease to run its course resulting in undue suffering with no scientific benefit. Mice are examined constantly for signs of ill-health. |

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Interventions against respiratory pathogens.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3"></td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[3]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[4]</td><td>Yes</td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Respiratory pathogens such as tuberculosis and influenza are leading global causes of death and disease. Tuberculosis was responsible for an estimated 8.8 million new cases worldwide and 1.77 million deaths in 2011. Similarly Influenza infection remains a global health priority with seasonal influenza epidemics affecting 5-15% of the population, causing severe illness in 3-5 million patients and approximately 250,000 to 500,000 deaths globally p.a.. The only licensed vaccine against TB, Bacille Calmette Guerin (BCG), is only partially effective and cannot be used in immunocompromised or HIV-infected people. Similarly, because of continually emerging new strains, more effective vaccines against influenza are also urgently needed. Both tuberculosis and influenza strains are developing resistance against the drugs that are used to treat them, so new and more effective drugs need to be developed. This project will play a critical role in the development of new vaccines and drugs against these diseases and accelerate their progression into human use.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">The models and the information resulting from the studies will be used to progress development and evaluation of new vaccines and drugs to combat diseases caused by respiratory pathogens. Evidence of the effectiveness of new vaccines or treatments will support their progress into clinical trial. Both these outputs will accelerate new interventions through to clinical application and effective interventions against</td></tr>
</table>

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

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| | respiratory pathogens such as tuberculosis, would be of colossal benefit to mankind. |
| What species and approximate numbers of animals do you expect to use over what period of time? | This project will use rhesus and cynomolgus macaques. Approximately 30 animals will be used each year. |
| In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end? | The protocols used in this licence have been classified as *mild* or *moderate*. We propose to evaluate vaccine or therapeutic strategies by challenging animals with the relevant pathogen. In unprotected animals this may result in infection and potential progression of disease. However, we have sufficient knowledge of our models to limit the level of disease that is required to determine whether a vaccine or therapy has been effective. The use of realistic low challenge doses, close monitoring of animals for clinical signs, the use of medical imaging such as CT and PET-CT all serve to allow early intervention before progression to severe disease. At the end of each experiment animals will be humanely killed so that the levels of disease in treated versus untreated animals can be accurately determined. |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | Whilst every effort will be made to use *in vitro* systems such as cell culture or use of continuous culture fermenters wherever possible, only in an animal model can we currently demonstrate efficacy in terms of alleviation of clinical signs and systemic pathology.<br>In order to study the complex inter-reactions between cells of the immune system during the course of vaccination and infection, it is necessary to reproduce as closely as possible this complex system in an experimental situation. Currently the only way to reflect this complexity and to define the dynamics of what is occurring in real time, is to use animal models that are reproducible and have been demonstrated to show the changes seen in man. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | Rigorous statistical systems will be applied to minimise the number of animals used whilst maximising the data gained from each experiment. A large archive of stored samples such as cells of the immune system, serum, plasma and tissues obtained from previous studies are available to be tested by new and improved assays as they emerge, reducing the need for further animals to be used. |

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| **3. Refinement**<br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | All work will be conducted with animals housed in social groups in enriched environments that allow natural behaviours. The application of imaging techniques and low dose challenge models will further reduce the length of studies and the potential for suffering.<br><br>Animals are sourced from UK breeding colonies where animals are well acclimatised to human presence and will be housed in social groups that allow them to groom each other and exhibit all their natural interactive behaviour.<br><br>Only the best new drugs and vaccines will be tested in the models in this project and all will have been shown to be safe and effective in other systems.<br><br>All procedures will be carried out by highly trained and experienced personnel and animals will be sedated for these to minimise stress. Vaccines and treatments will be given in the same way that they are to humans and blood samples to show that the vaccines have induced an immune response or that drugs are present at suitable levels, will be collected under sedation. The research establishment has extensive experience and understanding of the diseases being studied and animals will be monitored carefully for signs of infection and a number of clearly defined clinical observations are established that will predict those that will progress to disease. |

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Effect of incubation on post-hatch development</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Avian, incubation, muscle, myofibre, thermal</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">1</td></tr>
<tr><td>Purpose of the project (as in Article 5)[5]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Regulatory use and routine production</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[6]</td><td>~~Yes~~</td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">The aim of this project is to test the hypothesis that relatively small and short increases in incubation temperature have the ability to increase breast muscle fibre mass and that the effect is due as much, if not predominantly, to an increase in the number of fibres rather than an increase in the mass of individual fibres. By examining the period in embryogenesis when muscle fibre number is determined it is anticipated the necessary information to understand the relationship between thermal manipulations and hyperplasia will be obtained. It is also intended that the study will provide important information on so-called lean growth, lean growth being determined in part by prenatal increases in muscle fibre number.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">The benefits arising from this work include the advancement of our understanding of muscle biology and, for the poultry sector, provides a better understanding of the implications of temperature manipulation, either desired or inadvertent (as can happen in the centre of a stack of eggs when being incubated) during incubation.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td colspan="3">Gallus Domesticus, 1000 animals, 52 weeks</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</td><td colspan="3">No adverse effects are expected due to the thermal manipulations. Hatchability and liveability shouldn’t be negatively impacted. Animals remaining at the end of the trial will be euthanized humanely.</td></tr>
</table>

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

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| level of severity? What will happen to the animals at the end? | |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | The test variable affects the development of the whole animal. Therefore a cell culture cannot be used to test the effect of thermal manipulations on muscle growth. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | The variability of breast yield was assessed and used to calculate the necessary number of birds to obtain a valid measurement. No additional birds will be utilized. |
| **3. Refinement**<br>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. | **Chickens are an ideal species due to small size, low upkeep and previous research in this field have been done using chickens. The model was chosen as to minimize variability from effects such as hatch, batch or other blocking effects. The method of obtaining breast yield is a standard procedure, but coupled with histology offers validation to the effectiveness of making selections on early breast yield.**<br><br>**While the measurements needed are destructive, birds will be euthanized humanely prior to any data collection thus minimizing stress.** |

| Immunological control of autoimmune disease |
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| Type 1 diabetes, therapy, immune system |

- Summarise your project (1-2 sentences)

This project will identify new molecules to target therapeutically to prevent, or resolve, the development of in type 1 diabetes.

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

The immune system evolved to kill invading pathogens before they could damage our own tissues. In some individuals, the immune system attacks our own tissues, a feature called 'autoimmunity'. Our research is focused on determining the mechanisms by which the immune system (a) attacks our own tissues and (b) can be stopped from attacking our own tissues, to enable the design of new strategies to tackle so called 'autoimmune diseases'.

The immune system is a series of cells that have a specific job to do in the body. Some like B cells can produce antibodies that prevent a pathogen e.g. a virus or bacteria from attacking our tissues. Others, like T cells, are involved in killing our own cells that have become infected with a virus or bacteria. When B cells and T cells are made in the body, the immune system determines whether they have the potential to attack our own tissues that are not infected with a pathogen, and if they do, the immune system makes sure these B and T cells die. This is important to prevent damage to our own tissues that are not infected with pathogens. In some individuals there are genes that prevent the death of B cells and T cells that could kill our own uninfected cells leading to tissue damage. In type 1 diabetes, certain genes stop the B cells and T cells that could kill the insulin producing beta (β) cells in the islets of Langerhans from dying, and as a consequence, these B and T cells start to kill the β cells. This results in insulin deficiency. Unfortunately, although people with type 1 diabetes can control the disease with insulin injections, many sufferers develop life-threatening complications e.g. kidney failure and heart disease. Therefore, many investigators are trying to find a cure for type 1 diabetes. To develop a new cure, means we need to know many things: (1) what genes are involved; (2) what cells are involved; (3) how do the cells 'talk' to each other to push the B and T cells into damaging the β cells and (4) what other immune cells can stop this from happening?
Our Project aims to answer these questions. Unfortunately since the many complex interactions between cells that lead to β cell damage are unknown, we cannot reliably reproduce the process of β cell death in the test tube. For this reason, we use an animal model that spontaneously develops diabetes very similar to man. Using the model, we can take pancreatic tissue from animals humanely killed and look to see what types of immune cells are invading and attacking the β cells before diabetes develops. We can also isolate individual cells from the pancreas and compare the genes or proteins expressed by the same cells in animals genetically predisposed to developing diabetes versus animals that are not. By doing this, we may identify which genes are involved in the genetic pre-disposition to diabetes. We can test whether particular groups of cells that are responsible for stopping the activation of self-reactive B and T cells are defective by isolating them and testing their functionality in the test tube. In this way, we can identify key molecules and cells that could be a therapeutic target and design new drugs. Finally, by transplanting islets that have been modified to express a particular molecule into diabetic recipients, we can directly test the ability of

these modified islets to cure diabetes.

- Outline the general project plan.

Genetically modified and mutant mice are bred. There are several outcomes for these mice:

- They may be humanely killed, various tissues extracted and the immune cells present in these tissue analysed to determine the type of immune cell they are and their function.
- They may be given a new therapeutic treatment and the ability of the therapy to stop diabetes monitored.
- They may undergo a transplant (if diabetic) and following therapeutic intervention the ability of the new therapy to stop graft rejection and resolve diabetes will be monitored.

- 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.

Since we are interested in ex-vivo analysis immune cells prior to the development of type 1 diabetes, the majority of animals do not undergo any invasive procedures.

To establish if our therapies are effective, animals may be injected with a substance, irradiated to remove its immune system then subsequently receive a bone marrow transplant or may undergo an islet transplant if it is diabetic. These procedures have few adverse effects; transient pain and possible infection after surgery but this latter adverse effect is usually prevented by good surgical procedures and hygiene.

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

Type 1 diabetes is a disease that has no cure. Diabetic patients have a high risk of blindness, kidney failure, heart failure and amputations. This project will use animal strain that develop type 1 diabetes to identify new molecules that can be targeted to prevent or resolve diabetes and aid acceptance of islet grafts in diabetic recipients.

- 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.

12,500 animals will be used, but only 5800 will undergo any invasive procedures.

The non-obese diabetic (NOD) mouse spontaneously develops type 1 diabetes similar to man. We have learned a lot about the how the immune system goes wrong and attacks our own beta cells from analysing the immune system in NOD mice. It is therefore our model of choice.
More recently, ‘humanised’ mice, that is animals in which certain genes have been replaced with the equivalent human genes, or the mice can be transfused with human immune cells, has enabled us to study the effects of certain therapies for preventing type 1 diabetes progression in a more ‘human’ immune system. These ‘humanised’ mice are invaluable for identifying key therapies that have the best chance of working in man.

To make sure we use the minimum number of mice

- We cryopreserve strains we no longer need to prevent unnecessary breeding.
- We use state of the art technology to allow multiple parameters of immune cell phenotype to be analysed from small numbers of mice.
- We cryopreserve excess immune cells and use them to optimise the conditions of new experimental procedures
- Use the most up to date best practice in experimental design and implementation of techniques. Statistical advice is sought to make sure we use the minimum number of mice for statistically significant results.

- 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.

The immune system is complex, many cells and molecules interacting to destroy pathogens or our own tissues. Many of these molecules have yet to be discovered and as consequence we cannot accurately replicate the disease process in a test-tube.

However, we endeavour to use alternative approaches that do not use animals. For example, cell-lines can be used to test the effect of a new therapy on the target cell.

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

  The majority of our protocols do not cause any suffering to our animals other than perhaps transient pain from an injection. Surgical procedures are always conducted using best practice techniques, animals are kept warm and given analgesics. All animals are monitored carefully during and after surgery.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Defining and exploiting glycosylation in trypanosomes</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Trypanosome; glycosylation; drug targets; diagnostics</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)[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>We wish to continue our work to develop new, more affordable diagnostic tests and treatments for human African trypanosomiasis (“sleeping sickness”) and trypanosomosis in cattle. We shall study the detailed structure of complex carbohydrates (glycans) that are attached to proteins in the trypanosome parasite. We shall identify the enzymes that the parasite uses to make its glycan repertoire and assess them for suitability as drug targets. We also wish to discover where all the components of glycan synthesis take place in the trypanosome cell and how the process is controlled.</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 add to the knowledge of fundamental molecular biology and may lead to the identification of new drug targets and treatments for human and animal trypanosomiases. These are neglected diseases and our work will lead to better diagnostic tests for both the human and animal form of the 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 approximately 420 mice and 700 rats over the course of the five year 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>Animals are injected with parasites and blood sampled to monitor infection. Some animals that clear infections may be injected with a second parasite dose. The mice and rats may show signs of fever or lassitude over the next two or three days, but we monitor closely and animals are killed humanely at a fixed time point after infection, or earlier if they look ill for more than a short time. In</td></tr>
</table>

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

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| | order to recognise that they may feel ill for up to 12 hours due to the fever caused by the infection, the protocol is considered of moderate severity. |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | We have to use animal experiments to find out whether the parasite can survive the host immune system after an enzyme has been knocked out.<br>In addition, parasites kept in culture for a long time often lose features which are important for their survival in the host animal. For some structural studies large numbers of parasites have to be available at one time and this is not feasible with cultured cells. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | We use tissue culture-derived parasites wherever possible in our experiments and only transfer to animal work when it is needed for one of the reasons mentioned above. In addition, we have access to very sensitive analysis tools which reduces the amount of material and hence parasites, needed for structural analysis. |
| **3. Refinement**<br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | We use the mouse and rat infection models because they are predictable, well established and have made possible much of the work already done on trypanosomiasis. As explained above we use the animal model only after initial investigations using cultured parasites. We minimise the suffering caused to the experimental animals by using experienced personnel to ensure the animals are euthanized before the last stage of infection is reached. In this last stage the number of parasites in the blood is at its highest and the condition of the animals can deteriorate quickly. |

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Genetically altered rodent production in support of research programmes</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Production, genetically altered, Human disease</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)[9]</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[10]</td><td>Yes</td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>This project will create, breed and maintain rodents (mice) with genetic alterations showing desired human disease phenotypes (characteristics) and supply them for research into disease processes for the discovery and development of new medicines for the treatment and prevention of human disease.</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 use of live animals is needed in order to breed strains which mimic and show phenotypes (characteristics) typical of human disease in order to help develop new novel therapeutics for the treatment and prevention of human disease in areas such as Cancer and Respiratory diseases in order to improve patient’s lives.<br><br>The use of transgenic animals has become widespread and transgenic technology has evolved rapidly. Prior to the development of molecular genetics, the only way of studying the regulation and function of mammalian genes was through the observation of inherited characteristics or spontaneous mutations. Since the early 1980’s there has been rapid development in the use of genetically engineered animals as investigators have found an increasing number of applications for the technology.<br><br>Transgenic animals provide a means of evaluating genetic modifications in terms of anatomical and physiological changes in a complex system. Transgenic models are more precise in comparison to traditional animal models, for example the Oncomouse with its increased</td></tr>
</table>

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

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| | susceptibility to tumor development, thus reducing the course of tumor development in experimentally affected animals. |
| What species and approximate numbers of animals do you expect to use over what period of time? | We expect to produce approximately 4000 rodents (mice only) per year |
| In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end? | The procedures will not result in pain, suffering or distress to the animals and the effects of the genetic alterations will show mild or no adverse effects.<br><br>Animals produced under this licence will be transferred for use under the authority of other approved Project Licences within the company and to other sites UK & abroad, supporting work for human disease research of interest to the company. |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | The complex interactive processes of living mammals are not reproducible *in vitro* in the Laboratory and the possibility of using non-animal alternatives is explored by all research projects before the decision to use animals is taken.<br><br>As there are no non animal alternatives, the use of live animals is needed in order to breed strains which mimic and show phenotypes (characteristics) typical of human disease |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | Unnecessary animal production will be avoided by database searches to ensure the required strain is not already available and consideration to the use of tissues provided from established strains will be given prior to any new animal creation.<br><br>A centralised service is administratively efficient, with breeding controlled to produce batches of animals as needed and any spare can be made available for use by several different scientific research projects.<br><br>A reduction in the number of animals in breeding and produced will be sought by using the best and most appropriate method available to reduce wastage and produce animals of the correct genotype. As projects progress resulting in fluctuations in demand for transgenic animal usage then production breeding colonies will be reduced to a minimum to sustain the colony at a minimum |

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| | number of animals. |
| **3. Refinement**<br>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. | Rodents (mice) due to their short reproductive cycle time are the lowest sentient vertebrate group on which breeding for production of transgenic animals can be performed.<br><br>The mouse provides an appropriate species as the genome has been sequenced and can be manipulated, the required reagents are available and its basic biological and pathological processes are similar or identical to those in other mammals, including man.<br><br>The procedures will not result in pain, suffering or distress to the animals and where any surgical procedures are used appropriate pain relief will minimise any effects of the surgery and from our past experience the effects of the genetic alterations will show mild or no adverse effects |

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Vaccination against TB in murine models</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Bovine Tuberculosis, mice, vaccination, protection, Biomarkers</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></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>o</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[12]</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>Objective 1. Evaluate the efficacy of novel bovine tuberculosis vaccines.<br><br>Objective 2. Evaluate virulence of novel bovine tuberculosis vaccines.<br><br>Objective 3. Optimise tuberculosis vaccine administration protocols.<br><br>Objective 4. Immunological effects of abbreviating persistent BCG.<br><br>Objective 5. Identify immunological markers to enable assessment of vaccine induced protection (Correlates/Biomarkers).</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>1. Further understanding the development of T cell memory. The development of immune memory following vaccination is still a poorly understood area.. We have recently identified two independent mechanisms of protective immunity against TB in a murine model; one that appears to be dependant on medium term T effector memory cells, and one that is dependant on a yet unidentified long term central memory cell. We plan to further characterise these mechanisms which will have direct implications for a TB vaccine, and vaccination against bacterial diseases in general.<br>2. Identification of immune correlates (biomarkers) of protection. One of the</td></tr>
</table>

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

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| | great benefits of a murine model of TB vaccination and challenge is the unparalleled availability of reagents with which to probe and dissect the immune response. These advantages allow us to more easily identify immune correlates and biomarkers which have a significant role in either protection or pathology. We have identified pulmonary transcriptome RNA signatures using these methods and will further expand these studies to develop simplified protein-based methods of detecting components of these signatures.<br>3. **Use of the murine model to identify mechanisms of immunity in cattle**. Once identified, these markers can then be actively searched for in the bovine model, developing specific reagents where necessary<br>4. **ONE-HEALTH.** The cattle and human TB vaccine efforts are closely intermeshed and data generated in either system are useful and beneficial to the other. Thus, the results on vaccine efficacy and biomarker discovery undertaken under the authority of this license could have direct benefit to human health, and in particular the human TB vaccine development programmes. |
| What species and approximate numbers of animals do you expect to use over what period of time? | Mice (*Mus musculus*); approx, 8000 |
| In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end? | Expected levels of severity are moderate.<br>Adverse effects due to vaccination are mild, mainly local reactivity at injection sites.<br>Infection/challenge will lead to clinical symptoms of TB, although through the use of a strict comprehensive scoring system, animals will be euthanized before they show undue distress or suffering |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | There are no alternatives to testing of tuberculosis vaccines *in vivo*. There are presently no *in vitro* assays that can predict vaccine efficacy. To define biomarkers of protection, to predict vaccine efficacy without infection, one needs to study immunity following animal vaccination and infection. |
| **2. Reduction**<br>Explain how you will assure | Statistical assessment of sample size will be guiding every experiments and number of animals |

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| the use of minimum numbers of animals | used per group. This analysis will be based on previously published data, with professional statistical advice to be sought on a case by case basis. |
| **3. Refinement**<br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | Mice are the lowest vertebrate group which provide well characterised models and immunological tools for such investigations. We have validated that our murine models are relevant for predicting protection and immune responses in cattle.<br>To ensure the least severity possible, we employ a clinical scoring system which ensures that animals are euthanised as soon as they develop clinical signs of tuberculosis. |

**Project Title:**
Antibodies for research and assays
**Key Words:**
Antibody, Monoclonal, Neuromuscular, Diagnosis, Monitoring.
**Expected duration of the project (yrs):**
5 years
**Purpose of the project (as in Article 5):**
**Basic research:** Yes
**Translational and applied research:** Yes
**Regulatory use and routine production:** No
**Protection of the natural environment in the interests of the health or welfare of humans or animals:** No
**Preservation of species:** No
**Higher education or training:** No
**Forensic enquiries:** No
**Maintenance of colonies of genetically altered animals:** No
**Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**
To produce and characterise novel monoclonal antibodies for use in biochemical research. The protein targets against which the antibodies bind will be relevant to human diseases. New protein targets may be identified frequently as new discoveries are published in the scientific literature.
**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**
The monoclonal antibodies will be used in our own laboratories and in collaborating laboratories worldwide for basic research into human diseases, primarily neuromuscular disease, to improve diagnosis and to monitor potential therapies.
**What species and approximate numbers of animals do you expect to use over what period of time?**
We expect to use approximately 200 mice and 30 rats over a period of 5 years.
**In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?**
Adverse effects may possibly be moderate and may be sores/ulceration at the site of injection or haemorrhage at the site of blood withdrawal. Animals showing pain, distress and discomfort will be humanely killed (schedule 1). At the end of the protocol, animals will be either killed by a schedule 1 method or blood withdrawn by cardiac puncture under terminal anaesthesia.
**Application of the 3Rs**
**1. Replacement**
**State why you need to use animals and why you cannot use non-animal alternatives:**
Antibody production without the use of animals has not been shown to produce the range of antibody specificities and affinities required in this project. If a suitable non-animal technique becomes available, it will be used to replace the live animal work as soon as practicable.
**2. Reduction**
**Explain how you will assure the use of minimum numbers of animals:**
A minimum number of animals will be used for each antigen. Initially, we typically immunise 3 mice, though many other researchers use more than this. The 3 mice often show different immune responses. Occasionally, only one mouse will produce antibodies of the required kind. By experience we have found the initial immunisation of 3 mice with each antigen is a good compromise in that animal numbers are kept low, but there is a good chance of getting at least one animal with the required response within the time frame of the initial series of immunisations.
If a satisfactory mAb is already available commercially or on free distribution, we would not make new mAbs to duplicate it.

**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:**

Many different species-specific secondary antibodies are now available, but mouse is still the species of choice for monoclonal antibodies used in many applications.

Freund's complete adjuvant (FCA) is considered the gold standard for adjuvants by many immunologists and the general immunostimulatory properties of FCA have not been surpassed by any other adjuvant. Reaction to FCA will be minimised by using a newer formulation, minimising injection site volume and using the subcutaneous route.

Wellbeing, pain and distress of animals will be monitored regularly.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Mouse models of cancer progression and therapy</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cancer, melanoma, oncogene, metastasis, therapy</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[13]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></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></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Our aim is to improve the understanding of cancer initiation, progression and metastasis. Ultimately, by the use of our mouse models, we aim to develop new treatment strategies which can be translated into the clinic.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>This project will contribute to the understanding of tumour progression and metastasis mechanisms. Moreover, by understanding these mechanisms we will be able to tackle them in a therapeutic perspective; these animal models will provide more powerful methods to elucidate the underlying mechanisms of tumour progression and metastasis and to introduce drugs targeted to individual cancer patients.<br>In addition by assessing standard-of-care and new hypothesis-driven treatment approaches we aim to rationalise therapeutic decisions in the clinic based on the in vivo results from our studies. Finally, we expect to publish our work in peer reviewed journals thus sharing our findings with the scientific community.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Mice, around 30000<br><br>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</td><td>The majority of animals are not expected to show signs of adverse effects that impact on their general well-being. Very rarely the severity of these signs may be such that the humane end points may be</td></tr>
</table>

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

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| level of severity? What will happen to the animals at the end? | reached. The majority of the procedures will result in no more than transient discomfort and no lasting harm. All the mice will be humanely culled at the end point of the experiments. |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | The development of effective cancer therapeutics is an important goal of modern biomedical sciences. To identify potential cancer therapeutic targets, the processes involved in tumorigenesis must be understood at all levels, which requires the development of model systems accurately mimicking tumour progression. Cancer development is dependent not only on the changes occurring within the transformed cells, but also on the interactions of the cells with their microenvironment. The majority of our current understanding of carcinogenesis comes from the in vitro analysis of late-stage tumour tissue removed from cancer patients. In our lab, we perform a collection of in vitro assays to understand important points of tumour biology. While this has elucidated many changes experienced by cancer cells, it provides little information about the factors influencing early-stage cancer development in vivo. Also certain hallmarks of cancer, such as metastasis and angiogenesis, are impossible to study in vitro. Therefore, mouse models are important for studying the in vivo aspects of human cancer development. Transgenic mouse models have been engineered to develop cancers, which accurately mimic their human counterparts, and have potential applications to test the effectiveness of novel cancer therapeutics. This cannot be replaced by in vitro studies or different in-vivo models such as zebrafish or insects. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | Our use of in-vitro methods limits the number of animals required for the in-vivo investigation stage.<br>For our transgenic models we will use efficient breeding strategy to minimise the number of mice used to obtain the desired genotype.<br>The proposed experimental designs and methods of analysis of the results are always in agreement with statistical guidelines and with our bioinformatician scientist to provide meaningful data minimizing the number of animals used in each experiment. The design of individual experiments will generally involve factorial designs, which maximise the information obtained from the minimum resource. |
| **3. Refinement**<br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the | Mouse models that we are currently using faithfully recapitulate the human disease. Moreover, the mouse genome shares 98% homology with human genome.<br>We constantly work to improve husbandry and |

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| objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | procedures which minimise actual or potential pain, suffering, distress or lasting harm and/or improve animal welfare in situations where the use of animals is unavoidable. We ensure to provide the appropriate anaesthetic and analgesic regimes as well as appropriate humane methods of culling within animal facility. We ensure no visualisation of procedures in other animals and transport arrangements between facilities in appropriate containers. |

| Mechanisms of normal and malignant haematopoiesis |
| --- |
| Blood cells, leukaemia, chemotherapy, cancer genes |

- Summarise your project (1-2 sentences)

This projects aims to study the fundamental properties of the blood system and to elucidate the role of certain genes in determining these properties. We wish to gain a deeper knowledge of the mechanisms that mediate the development of the blood cell system and understand how it goes wrong in leukaemia (blood cancer), with the goal of developing and improving therapy.

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

The objective of our studies is to understand how normal stem cells in the bone marrow (BM) make blood and to identify factors that allow generation of cancer cells and cancer stem cells (CSCs). Better understanding of stem cell biology should allow us to find better treatments for leukaemia as leukaemia is initiated by CSCs. Common therapies do not kill CSCs allowing disease progression. CSCs have similar properties to normal stem cells. We aim to understand how normal and cancer stem cell functions.

- Outline the general project plan.

We will study the role of genes in leukaemia and in normal blood cell development. We will obtain blood cells from healthy humans or patients with blood cancers. We will also take samples from wild-type mice or mice predisposed to blood cancers. Cells will be studied using cell-culture methods and when necessary, cells will be injected into mice to study their properties. This work involves the introduction of mouse or human cells that may be manipulated to express or not express genes of interest into mice. Sometimes mice will be modified to remove genes of interest and tissues/cells will be studied. We will assess the response of the leukemia disease in the animal to clinically relevant chemotherapeutic regimes.

- 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.

Our work involves the introduction of mouse and human cells that may be manipulated to express the cancer genes into mice (whole animal bone marrow transplant). These mice are then monitored for abnormal blood cells development and leukemia. We then will assess the response of the leukemia disease in the animal to clinically relevant chemotherapeutic regimes. The expected adverse effects include the immunosuppression of mice after irradiation and therefore precautionary measures will be taken to reduce the risk of infection. Due to the fact that some of the cells being introduced are being studied for function in leukaemia development, particular attention will be paid to the appearance and general welfare of these mice, as the risk of developing cancers of the blood may be slightly elevated.

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

Key to our understanding of leukaemias and to the advance of new targeted therapies have been mouse models that mimic the disease process and pre-clinical studies of acute myeloid leukaemia (**AML**) and acute lymphoblastic leukaemia (**ALL**), the most common forms of adult and childhood leukaemia respectively. Therefore we use mouse models to gain a deeper understanding of the biology of blood cell development and leukaemia initiation/maintenance/progression, and as pre-clinical models to test established and novel compounds alone or in combination that could form the basis of future therapies.

- 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 have chosen mice to carry out our studies as the broad processes of blood cell development are well characterised and a wide range of reagents are available to address the biological properties of cells. Therefore these translational mouse models will allow us to determine human AML and ALL cell responses to novel therapies, to inform clinical trials. Over the course of this license it is estimated that 6500 mice will be placed on procedure. We will endeavour to reduce/replace the estimated mice numbers that are proposed for use in this project licence. The number of animals used in each protocol in this project is estimated based on my previous experience and power calculations taking into account the number of experiments needed and number of mice per experiment required to obtain the appropriate statistical power.

- 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.

While cell culture based studies can inform disease behavior in the animal (mouse and human), they cannot fully replace them due to the complexity of biological systems. However, we have generated systems that allow the *in vitro* manipulation of cells, thus enabling us to dispense with the generation of single and double transgenic mouse models for numerous molecules and replacing the need for lengthy breeding regimes to generate transgenic mouse models in the majority of experiments. This in turn reduces the number of mice required for some of the proposed experimentation. In addition, it is important to note that studies will be carried out in cell culture *in vitro* initially and only promising experiments will be carried forward to *in vivo* studies. An additional way in which animal numbers will be reduced is by imaging mice that are carrying fluorescently labelled cells. With this technology, fewer mice can be utilised per treatment as the same cohort of mice can be imaged for the time course of the experiment as opposed to relying on individual groups of mice per time point.

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

The protocols described will gain the maximal amount of scientific information from the minimal amount of mice, and the procedures are chosen to be the least invasive, thus minimising the suffering of the mice. For protocols involving bone marrow transplantation and leukaemia we have developed and successfully used a stringent distress scoring system which allows an immediate identification of mice with adverse effects. This system will allow us to efficiently minimise animal suffering.