Home Office

# Animals (Scientific Procedures) Act 1986

## Non-technical summaries granted during 2013

## Volume 34

## Project Titles and key words

- Measuring antibody memory against mutating viruses

  Influenza, Dengue, Vaccination, Antibodies

- DNA Editing in Neurogenesis

  NHEJ Brain VDJ Neurons

- Developing Therapeutic Biologics for Cancer

  Cancer, Antibody, Therapy, Model, Pharmacodynamic

- Brain mechanisms underlying cognition and emotion

  Primate, depression, anxiety, obsessive compulsive disorder (OCD)

- Mechanisms of B-cell cancer initiation and progression

  Immunity, Cancer, Lymphoma, B-cell, T-cell

- Peripheral and central mechanisms of acid sensing

  Neurone, acid, pain, respiration, anxiety

- Genes regulating tumourigenesis

  Cancer Genes Therapy

- Treatment of Inflammatory, CNS, Metabolic Disease

  Pharmacology, CNS, metabolic, inflammation, pain

- Neuropharmacology of Cognitive Processing

  Mouse, serotonin, GABA, cognition

- Regulation of neuro-developmental protective pathways

  RNA-binding proteins, stress, neural defects

- Molecular biology of cancer

  Cancer, Metastasis, Melanoma, Signalling pathways, Drug treatment

Measuring antibody memory against mutating viruses

Influenza, Dengue, Vaccination, Antibodies

- Summarise your project (1-2 sentences)

The overall aim of this project is to determine how the immune system responds to constantly mutating viruses like Influenza.

- 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.

A major problem with immunity against mutating viruses like Flu is that secondary exposure to a mutated virus often results in a poor antibody response which can lead to fatal illnesss and makes predicting the effects of flu vaccines and illness from flu infections difficult.

To design vaccines that offer protection against different virus strains will require an understanding of how the antibody response develops to cope with variant viruses, so that vaccines can be designed to work with the natural immune response to further enhance cross protection. To do this we will:

1. Make a panel of mutant/variant virus proteins
2. Use these in simple immunisations in mice to determine how the antibody response differs at different levels of protein variability
3. Synthesise these results to design test vaccines that offer improved protection against different viral strains.

- Outline the general project plan.

The project work is planned in 3 stages. Firstly we will improve molecular techniques for analysing antibody responses and then make a panel of mutant virus proteins. These will then be used for immunizing mice in simple vaccination protocols. Finally the antibody responses will be analysed by a variety of molecular techniques.

- 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.

All the protocols involve simple injections of purified proteins, sometimes with well-tolerated adjuvants, that are known to cause only transient mild discomfort. Occasionally we will be injecting genetically matched cells from one mouse into another mouse. All these procedures are well established and we expect few adverse effects.

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

By characterizing, with fine molecular detail, the antibody responses of mice to variant viral proteins we will gain a better understanding of how viruses like Flu cause disease in humans and animals, and how sub-normal antibody responses can be improved by the rational design of vaccines that provide cross-protection to variant viral strains.

- 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 plan to use up to 430 mice for this project. We have chosen mice for this project because their immune system is very similar to that of humans and they are well characterized for this type of study and so results obtained fit in a rich context of understanding and relevance. Animal numbers will be minimized by the use of a two stage exploratory-confirmatory experimental system where initially only small numbers of animals will be used per experimental group. When statistical significance is required for particular groups, only these group tests will be repeated using the minimum extra numbers of mice necessary to provide robust statistical analysis.

- 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.

It is necessary to use animals for the vaccination studies because the antibody response is the product of the whole complex of the immune system and this cannot be re-created in a test tube. Further, the results from animal vaccination map directly to the expected results in humans. For the first and third phases of the project we will only be using cell lines and human blood samples as well as molecular biology techniques. By using an exploratory-confirmatory experimental system we will minimise the numbers of animals used in each group and, further, will structure experiments so that we use the minimum number of control/non-test animals.

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

  The protocols in this project involve simple immunisations of mice using purified proteins and adjuvants, occasionally injections of cells, and occasionally sampling of small volumes of blood. All of these procedures involve brief injection or sampling using syringes with fine needles. These are well-established procedures and they are known to only cause mild transient discomfort. Animals will be inspected after injections and daily, and any animals that show the signs of anything more than minor and transient adverse effects will be humanely killed, although this is not expected.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>DNA Editing in Neurogenesis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>NHEJ Brain VDJ Neurons</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></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td>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>This project aims to understand the role of DNA recombination in neurones (cells in the brain), and to test if it is used as a way to encode long-term memory. This will be done through the detailed study of DNA obtained from neuronal cells of genetically altered mice. DNA recombination and repair is an important process in the normal biology of all cells, and is the process by which DNA is cut, deliberately or by damaging chemicals, and re-joined; without introducing any errors. In the immune system, this process creates the diversity seen in T and B cells, but in cells of the brain the purpose of DNA recombination and repair is poorly understood.</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 should ultimately provide important information on the role of DNA recombination in the development of neurones. In turn, this may enable a greater understanding of how disease processes such as Alzheimer’s affect memory storage and recall in the brain.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Mouse. 6000 animals 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</td><td>Animals will be bred in specific genetic crosses. Mice produced from these crosses are not expected to have any adverse and will be assessed regularly by trained staff and, when necessary veterinary experts, and culled if deemed to be in significant</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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| happen to the animals at the end? | stress or discomfort. At the end of the experiment animals will be killed. |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | The extremely complicated and complex networks of cells that make up the brain are impossible to mimic in cell culture. Clearly it is impossible to use samples from human beings, as it is sections of the brain that are used in this project. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | We have developed a method of working that maximises the amount of useful material collected from experimental mice and uses these collected samples rather than live animals in further research. This allows us to reduce the numbers of live animals produced for research purposes dramatically. |
| **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. | The mouse is the lowest vertebrate that can be used for this purpose with enough aspects of its genetics, anatomy and physiology shared with humans to generate biologically relevant data. It is important to use a mammalian system, with features of brain development and structure in common with humans.<br><br>Mice will be bred under strictly controlled environmental conditions in a state of the art establishment where they will have free access to water and food. Animals will be assessed regularly by trained staff and, when necessary veterinary experts, and culled if deemed to be in significant stress or discomfort. |

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Developing Therapeutic Biologics for Cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cancer, Antibody, Therapy, Model, Pharmacodynamic</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[3]</td><td>
<table>
<tr><td>Basic research</td><td>~~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>Cancer comprises a number of diseases that often have devastating consequences and depending on the types and stages of the disease, around half of all patients that develop cancer will die of their disease. Cancer therefore represents an area of high unmet medical need and we aim to develop new drugs to treat these patients. Although cancer research in the last 30 years resulted in the approval of several novel drugs that have extending the life of millions of patients, new medicines are still required to transform cancer into a chronic disease or more importantly cure patients. The aim of this project is to develop new and improved medicines (such as cancer immunotherapy) for the treatment of solid tumours. Our project plan involves all aspects of cancer drug discovery and development including developing new cancer models, testing how effective new drug candidates are and determining the best way to dose and combine novel drugs with existing treatments.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The work performed under this licence will allow the in vivo pharmacologists to generate key data around the safety and the anti-tumour efficacy of novel anti-cancer medicine. In addition, by analysing pharmacodynamics parameters, certain protocols described in the license aim to further understand the drug’s mechanisms of action at the tissue, cellular and molecular levels, in order to refine the translational potential of newly developed anti-cancer agents. Finally the benefit of this work will ultimately be for patients. Results generated</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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| | under the license will guide clinical development by determining how much and how often patients should receive the drug to maximise the therapeutic potential of the new treatments. |
| What species and approximate numbers of animals do you expect to use over what period of time? | For this project we will use mice as a model species. Numerous cancer models have been developed in this species and they have shown strong reproducibility generating key data that have influenced the clinical development for now approved cancer therapies. In addition the murine immune system is well characterised so this is highly relevant to develop novel medicine influencing the immune system. Animal models also allow us to understand cancer in the organ of origin or as it spreads throughout the body; this is important as when cancer spreads it is often fatal for the patient. Up to 35,000 mice may be used over this 5 year project. Typically this may support assessment of up to 20 new drugs and targets for cancer therapy. |
| 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 majority of animals used in these studies will experience minimal suffering. We ensure that as a result of the treatment/surgery, painkillers or anaesthetics will be used where required. We have expertise that allows us to closely monitor cancer progression including whole body imaging technologies similar to those used to monitor cancer patients. We will withdraw drug treatment from animals suffering adverse effects or humanely euthanize any animals that have developed advanced cancer to minimise unnecessary suffering. |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | Tumour biology is characterised by a complex interaction of tumour cells with stromal cell (immune cells, fibroblasts and vasculature). There is currently no in vitro system that can recapitulate all the complex cell to cell interactions and signalling feedbacks that occurs between the cancer cells and the surrounding stroma. However we will ensure that only a limited number of anti-cancer drugs are tested in vivo. This will be done by using drug candidates that have demonstrated efficacy for individual biological mechanisms using relevant in vitro biochemical and functional assays. Importantly, regulatory authorities such as the FDA and EMEA require compelling data packages to support the development of a novel medicine in humans. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | For each of our models we have accumulated a high content of historical data that help us understanding the variability within each experiment and between experiments. This information is now used in combination with statistical tools (e.g. |

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| | power analysis) to guide the design of our studies. Each of our experimental protocols comes with a “good statistical practice” statement that was approved by in house statisticians with whom we consult as necessary when planning in vivo studies. Finally we have characterised most of our routinely used models at the transcriptomic, genomic, histology and immune cell contents levels. This allows us to limit in vivo work only to relevant models. |
| **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. | The normal and tumour physiology of mice are well characterised and clearly contain strong similarity to their human counterparts. Recent genomic analysis confirmed that our genome is around 90% similar and 99% of mouse genes turn out to have humans orthologues, making mice a relevant model to test the safety and efficacy of novel anti-cancer medicines. In addition they are small, and therefore easy to handle. Our models will be of the minimal severity consistent with the objectives of the license. Tolerability studies on a small number of animals will be conducted for each drug candidate to ensure that there is no concern with the animal welfare. Importantly, best practice, for example the use of analgesics after surgical implantation of continuous delivery devices, will be employed to minimise suffering. |

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Brain mechanisms underlying cognition and emotion</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Primate, depression, anxiety, obsessive compulsive disorder (OCD)</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[5]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<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[6]</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 aim of this project is to identify those regions of the brain and chemical processes that underlie our normal and abnormal behaviour in a changing environment.</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 main benefit will be fundamental understanding of the physiological processes behind psychiatric disorders eg obsessive compulsive disorder, depression and anxiety with a view to informing treatments for these disorders, since current treatments are extremely limited.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Callithrix jacchus (common marmoset).<br>Approx. 415 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>Approx. 300 are expected to reach the moderate severity level and the remaining are only expected to reach the mild severity level.<br><br>None of the animals are expected to show signs of adverse effects that will impact on their general well being. However, animals on a moderate banding are expected to show clinical signs of a moderate severity during surgery to manipulate the brain and for abdominable implants and immediately post operatively.<br>Animals undergoing mild procedures are not expected to show anything more than transient discomfort and no lasting harm.</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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| | At the end, all animals on a moderate severity banding will be euthanased and data collected whilst animals on a mild severity banding will become breeders, be re-used or only rarely, euthanased. |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | It is not possible to investigate behavioural functions of the brain in a simplified experimental setting such as a tissue culture or in an artificial and biologically unrealistic computer simulation.<br>Existing techniques used in humans, including imaging of the brain, e.g.MRI scans, do not provide adequate information about the actual functioning of the brain. In addition, it is not possible to determine whether any changes in the brain that are seen in patients with various brain disorders are responsible for their actual symptoms. To determine this, specific manipulations of the brain are required to see whether they produce similar symptoms.<br>Such manipulations need to be performed in living animals. There is abundant evidence that much of the normal functioning of many of the brain systems is comparable across mammalian species. However, when studying advanced behaviour, both normal and abnormal, it is important to use non human primates in which those regions of the brain involved in the control of that behaviour is far more developed than those in the commonly used rodent model. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | Numbers are kept to a minimum by:<br>i). using a carefully controlled behavioural testing apparatus.<br>ii). using brain imaging to ensure correct targeting of the brain structure to be manipulated.<br>iii). using animals wherever possible as their own controls<br>iv). performing pilot studies before embarking on the main experiment.<br>vi). screening to ensure suitability of animal for particular study<br>vi). where appropriate, re-using animals from mild procedures. |
| **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 are using the most minimally invasive procedures to achieve the objectives of this project and we only apply new technology in primates once it has been validated in rodents by our collaborators. When marmosets are used, whenever possible they are tested on the same behavioural tests as used to test intact and brain damaged humans to maximise the ability to extrapolate findings from monkey studies directly into the clinical setting.<br>The laboratory has over 2 decades of experience in this research and all experiments are performed in |

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| | close interaction with the named vet and named animal care and welfare officer that are in daily contact with the animals. All procedures are carried out by experienced staff and all procedures are continually reviewed and outside advice is sought appropriately to ensure best practice and high standards of animal welfare. |

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Mechanisms of B-cell cancer initiation and progression</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Immunity, Cancer, Lymphoma, B-cell, T-cell</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[7]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></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[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>We aim to investigate the molecular mechanisms of B-cell cancer pathogenesis through the development of bona-fide pre-clinical mouse models of human disease. In particular we are interested to investigate how conditions associated with cancer development and intimately connected with the immune system such as inflammation, autoimmunity and infection contribute to the development of B-cell cancers.<br>The proposed work uses cancer mouse models of pre-clinical relevance, to provide information on genes and immune conditions key for cancer initiation, on the risk of cancer progression, and resistance to therapy. We aim in addition to understand how cells of the immune system interact with pre-cancer cells and cancer cells. This information may warrant the definition of how and when to therapeutically intervene to enhance anti-cancer immunity and control cancer growth.</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 the work can be summarised as follows:<br>1. We will gather a deeper knowledge of the pathways, genes and immune conditions key for cancer initiation and progression; identify causal mutations in cancer; and to integrate this knowledge with the role of immune system in cancer formation and progression.<br><br>2. The information gathered in these studies should allow a better definition of the risk of cancer formation, progression and relapse</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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| | and assist the identification of biomarkers and development of novel and effective therapies, relevant for the design of future cancer treatments by the pharmaceutical industry.<br><br>3. The cancer models developed in these studies will be of pre-clinical importance and of value to other scientists for the development and testing of anti-cancer therapeutics, including therapies aiming to stimulate our own immune system to recognize and eliminate cancer cells. |
| What species and approximate numbers of animals do you expect to use over what period of time? | We will use only mice in this project and a maximum of 10,000 animals will be used per year over the 5 year term. |
| In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end? | Mice will be bred to generate specific combinations of mutations of both mild and moderate severity. Although the majority of mice will not show any adverse effects, phenotypes may be exacerbated upon administration of substances with transgene altering properties. Other mice may per se be expected to develop spontaneous tumours.<br><br>We will assess phenotypes in the mouse both under steady conditions and upon immune challenge. The immune challenge includes one of the following situations: mimicking of an immune response by immunisation, in which antigen is administered through various routes; infection with a live pathogen. For the majority of cases no significant adverse effects are expected and the most likely common reaction will be fever leading to signs of distress. If any of these signs are not resolved the mice will be culled.<br><br>As mice are expected to eventually develop tumours, they will be closely monitored following NCRI guidelines and culled at signs of persistent discomfort or if the tumour size reaches the recommended guidelines.<br><br>All animals will be monitored closely and will be humanely killed if unexpected ill health occurs, if severity limits are approached or if scientific objectives have been attained. |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use | While valuable studies of human cancer are performed using tumour material, the mechanistic |

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| animals and why you cannot use non-animal alternatives | understanding of cancer pathogenesis requires the use of living animals. In particular the development and function of the immune system involves many different cell types interacting in a dynamic three-dimensional environment. Similarly, cancer development and spread involves a plethora of interactions between cancer cells and their surrounding cells, governed by multiple signals originating from both their immediate neighbours and from distant tissues.<br><br>The study of cells in culture provides us with cues on the mechanisms of cellular processes in a simple and defined context, which allows the establishment of hypothesis of the function of cells in an intact animal. However, these systems do not recapitulate the complex cellular interactions described above. In particular the culture of GC B-cells, from which most of the B-cell malignancies are derived is currently not possible. Therefore the use of animals is essential. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | We will collect as much evidence as possible from current literature, and through the analysis of available human cancer data. These analyses will be, whenever possible, confirmed using primary patient material. We will also perform studies in vitro using established cancer cell lines and mouse primary non-transformed cells. These studies will precede and guide the generation of relevant transgenic mouse models.<br><br>The breeding of transgenic animals will be reduced through collaborative access to strains. We will avoid overbreeding, and lines under sporadic use will be maintained at low levels, and frozen whenever practicable, and/or maintained in collaboration with other licences to minimise redundant breeding.<br><br>The proposed experimental designs and methods of analysis will be discussed with members of the laboratory, and those of our collaborators, and we will seek additional advice from the statisticians employed by our Institute.<br><br>We will perform pilot experiments in which a small number of animals per group are used for genotype comparisons. Depending on the results obtained from pilot studies we will then proceed to perform larger cohort studies to determine if the observed difference is statistically significant.<br><br>Further we will use whenever possible modified bone marrow cells for the reconstitution of the |

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| | immune system in host animals, which permits the increase of sample measurements together with the reduction of the breeding of transgenic animals. This approach also allows bypassing complex genetic crosses aiming to identify intrinsic versus extrinsic phenotypes.<br><br>When performing tumour studies we will, when possible, follow up tumour development using whole body scanning as it allows a reduction of the number of animals used. This strategy goes along with the concept of not using more animals than the ones essentially needed to obtain informative and statistically significant data, and to maximise the data obtained from each animal, e.g. by studying the effect of mutations in multiple cell types. |
| **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. | The mouse is one of the model organisms which most closely resembles humans and its genes are functionally conserved. Of particular relevance to answer the scientific questions in this project mice have genes of the immune system not represented in other non-protected animal model organisms like the nematode worm and fruit fly.<br><br>Mice can be genetically altered, there is extensive literature concerning the topics of our investigation, and our own studies can be enhanced by combination with many complementary models developed by others in the field.<br><br>We will strive to generate transgenic mice in which mutations are induced specifically and conditionally in the cell population of interest, using for the effect e.g. Cre-LoxP conditional alleles or alleles, which function can be activated or terminated through the use of Tet-On and Tet-Off systems. These mice should not display a phenotype until the mutation in the candidate gene is induced.<br><br>In all our experiments we will set humane endpoints and write an experimental protocol, which will include details of possible adverse effects.<br><br>When administering substances or cells to animals, the route used for delivery will be such as to achieve “best practice”, that is to minimise or avoid adverse effects, while minimising the number of animals used, and maximizing the quality and applicability of results. For that reason we propose in this project licence a variety of routes of administration of substances and cells to achieve the scientific objectives, while minimizing the waste |

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| | of animal’s lives. |

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Peripheral and central mechanisms of acid sensing</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Neurone, acid, pain, respiration, anxiety</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></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>Acid can activate neurones in a wide variety of contexts, for example, the tissue acidosis characteristic of inflammation and carbon dioxide induced acidosis that regulates breathing. The molecular mechanisms by which neurones detect acid, and the precise identities of the neurones involved in acid-driven sensations/behaviours are currently unknown.</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>By identifying the molecular mechanisms of acid detection, as well as the neuronal subsets involved, this research will lead to fundamental advances in our knowledge of neuronal function and could, in the long term, aid the development of new therapies to treat pain, disordered breathing syndromes and conditions associated with respiratory imbalances, such as panic disorder.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>5300 mice and 210 naked mole-rats are estimated to be used over a period of 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>Many of the procedures detailed in this proposal will be undertaken using general anaesthesia to limit pain/distress. Some experiments will involves short-term pain, similar to that experienced by the splashing of lemon juice into cut skin. Small groups of animals will develop a form of arthritis, or experience fluctuating oxygen and carbon dioxide levels, both of which will involve a moderate level of</td></tr>
</table>

[9] Delete Yes or No as appropriate.

[10] At least one additional purpose must be selected with this option.

| | |
|---|---|
| | pain and distress. All animals will be killed at the end point of each part of the study. |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | This work will examine complex behaviours such as pain and breathing control. Cell lines and *in silico* methods do not provide in depth information of complex behaviours that are the result of complex neuronal circuits functioning at the whole organism level and thus it is necessary to use animals. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | Statistical analyses will be employed to ensure that the minimum number of animals will be used, as is necessary to produce statistical useful results. Cell lines will be used in place of freshly isolated neurones to examine the structure and function of any proteins of interest that we identify in this study. |
| **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 being used because there are good animal models for pain in this species and it is the mammalian species best developed for using genetically altered animals, thus allowing analysis of genes of interest in animals lacking that gene. Naked mole-rats are being used because they display an insensitivity to carbon dioxide/acid that is unique among mammals. By comparing mice to naked mole-rats we can identify the molecular basis for the difference in naked mole-rats and thus learn more about chemosensing in other mammals, including humans. Welfare costs to the animals will be minimized through use of: aseptic technique, analgesia when required and appropriate and termination of an animal's life if necessary to prevent further, unnecessary suffering. |

| Genes regulating tumourigenesis |
| --- |
| Cancer Genes Therapy |

- Summarise your project (1-2 sentences)

  This project aims to identify the genes that cause cancer and to develop new ways of treating the disease.

- 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.

  Despite the advances in cancer research and the treatment of cancer a number of critical questions still remain largely unanswered: (i) which genes drive the development of cancer, (ii) what genes determine whether a cancer patient responds well to therapy and (iii) how do we use genetic information to improve the way patients with cancer are treated? For example, DNA sequencing studies have shown that mutations in genes such as *ARID1A* are found in patients with breast, ovarian and oesophageal cancers; at present the reasons for why these mutations cause cancer are largely unknown. Perhaps more importantly, we do not know how to effectively treat cancer patients who have *ARID1A* mutations. The work outlined in this application is aimed using mice to model human cancer that is caused by genes such as *ARID1A* and using these mice to identify better ways of treating cancer patients.

- Outline the general project plan.

In general, the approach we will use is as follows: (1). The identification of cancer genes using a computational approach as well as laboratory-based experiments (2) the generation of genetically engineered mice that have cancer gene defects (3) the testing of novel therapeutic approaches to cancer using these mice.

- Predicted harms: Give a brief description of the procedures to be applied to the animals used in this project and describe the expected adverse effects.

  - The procedures to be used in this project include:

    - Stimulating egg production in mice
    - Minor surgery and injections in mice
    - Giving mice drugs
    - Injecting mice with tumour cells to form tumours in mice.

  - The predicted harms include:

    - Infection due to minor surgery and injections
    - The deleterious effects of having tumours
    - The side effects of drug administration

- To minimise these predicted harms, we will:

    - Use anaesthesia and pain relief where appropriate where appropriate
    - Closely monitoring animal behaviour and well-being to identify where and when adverse effects occur
    - Ensure that mice with tumours are effectively monitored so that their tumours do not grossly impair their health
    - Use drug treatments that are designed to minimise harmful effects

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

Our aims are to design better ways of treating cancer patients, so that they live longer and so that their quality of life is improved. To do this, we need to identify better ways of targeting cancers without also having a harmful effect on normal cells. The work that we propose in this application is designed to identify these more effective treatment approaches and thus will have obvious benefit to patients. Alongside this clear advance, our work is also aimed at increasing our understanding of how genes control cancer. This work will in the future help others to better understand the disease and again, will ultimately lead to better treatments for the disease.

- 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 will only use mice in this project. Our estimates are that the work will involve the use of 18,500 mice over the next 5 years. In terms of reducing the number of animals, we will design each experiment so that we use only the number of animals that mathematically allows us to discriminate the response between, for example, drug treated and non-drug treated groups of mice.

- Demonstration of compliance with the 3Rs: State why you have to use animals and cannot use non-animal alternatives. Where appropriate, say how you will use non-animal studies in parallel with the project.

Animal models are required to fully recapitulate the properties of human tumours in cancer patients. These properties cannot be adequately recapitulated in the laboratory. Similarly, the effects of drugs need to be tested in live animals so that their effects on the normal cells can also be assessed. Nevertheless, alongside this animal work, we will continue to use laboratory experiments to model how tumour cells respond to drugs and how specific genes alter tumour cell behaviour. These laboratory experiments will enable us to reduce the number of mice we use and will allow us to focus our animal experiments on those projects where animals are only absolutely required.

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

Each of the protocols highlight the potential adverse effects that could occur as a result of the procedures used. For example, we have detailed the maximum tumour sizes that will be used before mice should be sacrificed. We will also use anaesthesia and pain relief where appropriate. Also by closely monitoring animal behaviour and well-being will identify where and when adverse effects occur. We will also ensure that mice with

tumours are effectively monitored so that their tumours do not grossly impair their health. Where we use drugs, we will use drug doses that are predicted to not elicit adverse effects.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Treatment of Inflammatory, CNS, Metabolic Disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Pharmacology, CNS, metabolic, inflammation, pain</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></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[12]</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 overall aim of this research program is to generate potential medicines to treat diseases including Schizophrenia, Alzheimer’s Disease, metabolic diseases such as diabetes and obesity, pain and inflammation. These therapeutic areas are those in which there are either no current treatments or the existing therapies do not work fully or have side-effect complications.<br>Through a process of chemical synthesis and testing in cells, tissues and animals, candidate drugs will be discovered and eventually progress to clinical trials to ultimately have the potential to offer novel effective treatments in people.<br>The work conducted under this licence will continue that carried out under a separate licence that identifies new drug targets via analysis of physiological and behavioural characteristics of mice in which individual genes identified as druggable from human and mouse genome databases have been disrupted.</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 benefits of this project relate to the discovery of new chemical entities that could lead to more efficacious and safer medicines for pain, inflammatory, neurological and metabolic disorders. The work conducted under this licence will provide novel information on the identification and characterisation of new chemical entities acting through novel targets and/or mechanisms. The treatments will have superior efficacy or better safety profiles and several will represent first in class drug discoveries in areas of significant unmet medical need. In the case of pain and some CNS disorders the benefit will be symptomatic relief and</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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|---|---|
| | improved quality of life for patients. In the case of metabolic disease, neurodegenerative CNS disorders and certain inflammatory conditions it is expected that life-expectancy will also be prolonged |
| What species and approximate numbers of animals do you expect to use over what period of time? | Rats and mice. A maximum of 17000 rats and 23000 mice over 5 years |
| In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end? | The majority of animals (>95% of those used on the license) are not expected to show any adverse effects. The most likely adverse effects are those related to the in vivo administration of novel compounds and may be of mild severity. Very rarely, a moderate severity limit may be reached. Overall, approximately 90% of animals used on the license will be within the mild severity and 10% moderate severity. At the end of use, animals will be humanely killed. |
| **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 current in vitro alternatives which would model the complex in vivo processes involved in absorption, distribution, metabolism and excretion or safety of candidate drug molecules. Similarly, there are no in vitro models for conditions such as pain that involve integrated responses from tissues including the brain, spinal cord, peripheral site of injury and circulating mediators. As such in vivo studies are essential.<br>There are alternative strategies that can be used, particularly for studies investigating cellular responses to target manipulation by compounds, where in vitro studies can be carried out on cultured or primary cells. We will also use ex vivo experiments were possible, for instance, using tissue samples to assess the levels of inflammatory markers or brain slices to measure electrophysiological responses to test compounds. For metabolic projects, ex-vivo assays using human colon tissue and adipocytes can be accessed and can be used before compounds enter in vivo testing. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | We can consult with statistical experts to help with experimental design. We have access to specialised statistical packages to ensure that data can be analysed with rigour. Optimal animal numbers will be determined based on experience from studies carried out under previous licenses, accrued historical databases, or published studies. Meaningful biological change and measurable outcomes will be defined and estimates of biological variability will be used in sample size and |

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| | statistical power calculations. The tracking of changes in variability over time and power analysis may allow animal numbers to be further reduced |
| **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. | Species choice is mouse or rat and will be made based on the end-point employed (i.e. whether more robust in mouse or rat) and knowledge of the target. The overall premise of the license is that the most refined model possible will be used at each stage of the work plan i.e. the greatest number of compounds will be tested in models of minimum burden on the animal (duration, stimulus required for experimental window and end-point). This is the major influence on model or method choice. Animal numbers on moderate severity protocols are limited to ensure this. If animals show clinical signs such as a particular level of weight loss or coat changes associated with the severity limit of the protocol they will be humanely killed. |

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Neuropharmacology of Cognitive Processing</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Mouse, serotonin, GABA, cognition</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[13]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></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>Most complex animals, including humans, live in a constantly changing world. What holds today often changes in the future, and an animal should therefore be able to respond to its environment in a cognitively flexible manner. This project will study the brain mechanisms that underlie cognitive flexibility in mice. The project is likely to be of value because loss of cognitive flexibility is characteristic of several common and debilitating psychiatric disorders in humans including schizophrenia and Alzheimer’s disease. Knowledge of the specific brain mechanisms, especially relevant neurotransmitters, is likely to help in the design of drugs to treat such disorders.</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>Successful completion of this project should result in two types of benefit. First, it will advance our knowledge of the psychological mechanisms that underlie cognitive flexibility. We have already developed a more advanced theoretical perspective that will be tested by the experiments performed here and the later studies will help to specify the relevant brain mechanisms. The data will be of value to both psychologists and neuroscientists studying learning and memory. The second benefit will be longer term and will lead to more rational design of drugs for the treatment of schizophrenia and other disorders.</td></tr>
<tr><td>What species and approximate numbers of</td><td>The great majority of our experiments will use mice. We expect to complete up to 30 experiments within the project, which would</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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| animals do you expect to use over what period of time? | equate to a total of about 1500 mice with some additional animals being used solely within a breeding programme for genetically manipulated animals. We may also use rats for a small number of experiments with total usage not exceeding 200 – 300 animals. |
| In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end? | The project will use relatively simple learning procedures in which, after an animal has learnt a task, the rules for correct performance are changed or fully reversed. Analogues of such tasks are often used in in human diagnostic procedures (e.g. the Wisconsin card sort task). Our tasks will depend on reward rather than any form of punishment and are likely to involve minimal levels of stress. In later studies we shall use methods that allow us to identify the particular brain structures that contribute to performance of our tasks. These include methods that allow us to visualise areas of brain activated during the learning task and to manipulate the function of that area. Experiments of the latter kind will involve a surgical manipulation. We shall use procedures that are very similar to those employed in human neurosurgery including gaseous anaesthesia, analgesia during and after the procedure and housing conditions that will promote full and uneventful recovery. Overall we anticipate very few adverse effects for the animals used in this project. The animals will be humanely euthanized at the end of an experiment. |
| **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 animals in order to be able to study behaviour and cannot use humans because appropriate drugs and other relevant procedures are either not available or are ethically unacceptable. We shall use rodents, mainly mice, because such tasks are easy to implement and because the general details of brain structure and function are already well understood and are sufficiently similar to humans to allow extrapolation. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | Behavioural experiments of the type described here typically involve several groups of animals experiencing slightly different learning conditions. Group sizes are of the order of 8-12 giving a typical total of 36-48 in an experiment. We use statistical procedures (power analysis) to minimise group size while ensuring that we are still likely to obtain statistical significance when an effect of interest is present. |
| **3. Refinement** | Rodents show complex and flexible learning |

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| 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. | that depends on sufficiently similar brain strictures to those in humans to allow a reasonable measure of extrapolation. Mice allow the possibility of genetic manipulations of brain neurotransmitter systems that are a valuable complement to pharmacological studies. High quality housing with appropriate enrichment, avoidance of single housing wherever possible and the avoidance of tasks that depend on punishment will help to minimise welfare costs. |

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Regulation of neuro-developmental protective pathways</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">RNA-binding proteins, stress, neural defects</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[15]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[16]</td><td>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 colspan="3">Neurological diseases causing intellectual disability (ID) have a worldwide prevalence of around 2%. The most common genetic conditions causing ID are Down syndrome, Klinefelters’s syndrome and Fragile X syndrome amongst many others. ID can be triggered by the dysfunction of cellular pathways that normally detect suboptimal environmental conditions and help neural cells to survive when exposed to external stress. Absence or mis-regulation of these protecting factors can cause cellular stress even in the absence of external stimuli leading to increased cell death. Changing the tightly controlled balance of cell survival and death towards death in an organ is particularly deleterious during development. The developing brain may not have sufficient cells or the right cell populations to generate all neural lineages of the brain and remains under-developed. Our aim is to identify novel factors that can protect the developing and adult brain from external factors causing cell stress. We then test whether the identified factors can provide protection from cell death when the normal protective pathways are impaired.</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">Our projects will improve our current understanding of how a healthy organ is established and maintained. We will further address the question how neuro-developmental disorders are established during development and whether we can prevent neurological disorders by modulating the cellular stress response pathway.</td></tr>
</table>

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

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| What species and approximate numbers of animals do you expect to use over what period of time? | Mus musculus. To generate the transgenic lines we will need 250 mice. For our experiments we will use 1600 animals over the duration of this licence (5 years). To get this number of animals we will have to breed 3400 animals (total 5000). |
| 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? | When analysing the functional relevance of our candidate genes in the brain, we are expecting clinical signs of moderate severity. We expect clinical signs of gait problems, ataxia and cognitive defects, such as anxiety. When we test potential drugs to protect neural cells from cell death, side effects of the drugs may occur in the form of weight loss, liver dysfunction and algesia. The mice will be culled as soon as the mice show any of these signs. |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | At present there are no cell culture systems that models neuro-developmental disorders or complex developmental stages of the brain. Any potential drug to modulate RNA methylation pathways will be first tested in neural culture systems. However, cell culture systems do not provide all cell lineage of a developing or adult brain. Therefore, it is still essential to test the potential modulators of stress pathways in their native environment. To ensure that the same mechanisms apply for humans it is then essential to validate our results in human cells. We always consider the use of alternative techniques to answer our research question and use organotypic in vitro culture systems whenever possible and available. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | The experimental design and estimated number of animals used in the proposed studies will be based on Festing et al. (2004). '*The Design of Animal Experiments – Reducing the use of animals in research through better experimental design*'. Laboratory Animal Handbooks No. 14. The Royal Society of Medicine Press limited. When designing the experiments we perform statistical analysis, such as power calculations to ensure that we use the minimum number of mice per group that will be informative. Using well-established agents and assays will reduce the number of animals. When we test a compound, such as a small inhibitor or peptide, the drug will be pre-screened to obtain an indication of the minimum dose that is likely to be effective. To maximise the information from a single animal, we will collect samples from all body sites showing the expression of the enzyme. Other tissues than brain that might be affected are skin, mammary gland, thymus, trachea, intestine, lung |

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| | and others. We try to reduce the number of animals by for example planning experiments together with colleagues working on similar scientific questions in order to share samples and to obtain the most information out of the least 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. | Mice are evolutionary the lowest form of mammals that can be used to study brain biology and they are the only animals in which the transgene technology works reliably.<br><br>By only using well established and extensively described methods and protocols we minimise the uncertainty of how compounds affect the wellbeing of an animal. To reduce the harmful effect of a candidate gene we will use models that allow regulation of the activity of the gene under study. The mice will then be closely monitored. Other models will only be chosen when there is sufficient evidence from cell based assays and publications that the candidate gene does not induce a harmful phenotype. |

| Molecular biology of cancer |
| --- |
| Cancer, Metastasis, Melanoma, Signalling pathways, Drug treatment |

- Summarise your project (1-2 sentences)

The aim of our work is to understand the cell signalling pathways that determine tumour cell growth , survival and metastasis and identify novel therapeutic targets that can be used to treat cancer.

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

Cancer is a disease of the genes, every tumour contains multiple genetic changes (mutations); how most of these mutations contribute to cancer development, progression and metastasis is not fully understood. In cancer, the development of metastatic disease signals a poor prognosis. Tumour metastasis consists of a series of discrete biological processes that involves invasion of the tissue surrounding the primary tumour, intravasation of the tumour cells (shedding and survival in the circulation) extravasation into a distant organ and growth at the new (secondary) site. We believe that blocking cell migration and invasion could be effective in some cases of metastatic cancer.

The objective of our work is to identify genes and the dysregulated signalling pathways involved in cancer with particular relevance to malignant melanoma and pancreatic cancer, and to understand how these genetic changes give tumour cells the ability to metastasize.

- Outline the general project plan.

The objective of our work is to understand the cell signalling pathways that determine tumour cell proliferation, survival and metastasis. We will achieve this objective through a programme of studies:

1. By generating genetically modified mice of genes that have been identified as having a role in cancer and metastasis.

2. To evaluate the behaviour of genetically modified cells in tumourigenesis and metastasis.

3. To test the effect of novel and existing therapeutic agents on the treatment of tumours and metastasis arising in our model systems.

- 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.

Genetically modified mice carrying specific genes will be generated, tumours and/or metastasis may develop in these mice, the mice will be monitored using non-invasive imaging methods; at pre-clinical end points, the mice will be killed humanely and the tumours and metastasis analysed at post–mortem. These mice may also be treated with novel therapeutics and the effect of treatment assessed by non-invasive imaging methods. Genetically modified cells may also be inoculated into immunocompromised mice in order to analyse the effect of the modification on tumour development and metastasis.

The expected adverse effects are tumour growth and the development of metastasis,

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

This work is expected to provide novel information on the role of target genes in tumour formation, progression and metastasis as well as identify novel therapeutic targets that can be used to combat cancer, in particular the spread of cancer.

- 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.

Only genetically manipulated animals will be used in this project, since only in mice is the technology sufficiently advanced to allow us to generate genetically modified mice.

- Demonstration of compliance with the 3Rs: State why you have to use animals and cannot use non-animal alternatives. Where appropriate, say how you will use non-animal studies in parallel with the project.

The process of cancer requires complex cell-cell and cell-stromal interactions, which can only be modelled in animals. Although a great deal of our work involves the use of cultured mammalian cells to study the effect of a given genetic change on cell behaviour, these experiments are limited by our inability, as yet, to recapitulate all the interactions required for tumourigenesis.

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

Mice will be housed in cages with sterile bedding food and water. Trained competent personnel with experience of pre-clinical models of cancer and who are familiar with the effects of anti-cancer drugs on rodents will perform all procedures. Advice will be sought from statisticians and studies will be designed to use the minimum number of mice. Mice will be inspected daily. Anaesthesia and analgesia will be used to minimize stress and suffering.