Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 23

Project Titles and key words

- Neural Systems and Circuits for Binocular Vision

  Cortex; stereoscopic vision; MRI; neurophysiology

- Pathogenesis of murine osteoarthritis

  Osteoarthritis, cartilage, disability, joint replacement, pain

- Targeting angiogenesis to improve stem cell transplantation

  Stem cells, transplantation, cancer

- Develop antibody drugs for cancer

  Cancer, drug, antibody, research

- Brain co-ordination and vital behaviours

  Brain, hypothalamus, sleep, alertness, body weight

- Regulation of Immune Response in Rodents

  immunoregulation; infection; inflammation.

- Development & homeostasis of the vertebrate nervous system

  Central, peripheral, enteric nervous system, embryogenesis, signalling

- Patterning embryos with genetic oscillations

  Embryology, scoliosis, biological clocks

- Molecular switches in inflammation

  Arthritis, Asthma, Macrophage, Inflammation, Molecular switch.

- Production of antibodies: DNA replication research

  Antibodies, Antigen, Immunisation

- Optimizing pharmacotherapy based on PK principles

  Pharmacokinetics, Pharmacodynamics, Lymphatic transport, Biodistribution

Neural Systems and Circuits for Binocular Vision

Cortex; stereoscopic vision; MRI; neurophysiology

5 years

Basic research	**Yes**	~~No~~
Translational and applied research	~~Yes~~	**No**
Regulatory use and routine production	~~Yes~~	**No**
Protection of the natural environment in the interests of the health or welfare of humans or animals	~~Yes~~	**No**
Preservation of species	~~Yes~~	**No**
Higher education or training	~~Yes~~	**No**
Forensic enquiries	~~Yes~~	**No**
Maintenance of colonies of genetically altered animals[1]	~~Yes~~	**No**

Binocular vision gives us a sense of depth; it can tell the brain how far away objects are, what size they are and where one object sits in relation to another. We are now improving our understanding of what actually happens in the brain when we see things in depth. Whilst solving this puzzle is an interesting problem in itself, it turns out that binocular vision often goes wrong during development.

About 1 in 50 people have a problem with their binocular vision and there is a large programme of clinical intervention, including surgery on the eyes in children. All of this implies a large health care cost and a significant burden of disease, partly because these problems are so common.

Although we work with human brain imaging, investigations with animals are also part of our research strategy. Here we can examine directly the neural signals and circuitry that are responsible for binocular depth. This basic research will be a platform for any future improvements in clinical practice. The aim is to characterise functionally and structurally neural mechanisms underlying the integration of information for binocular depth perception at critical stages of cortical processing along the ventral visual pathway.

The proposed research will (a) use animal models to increase fundamental knowledge about the brain mechanisms underlying binocular performance and (b) transfer knowledge of binocular vision to the understanding of human disorders and possible treatments. No equivalent programme of work is under way, within the EU or elsewhere.

Eight rhesus macaque monkeys (*Macaca mulatta*) will be used in two groups of 4, each group being used for about 4 years in total.

The animals will be trained to carry out tests of binocular vision. They will be introduced to the tests slowly and progressively. Initially they will be trained with **'**treats**'** but to be sure that the limits of performance have been explored, the animals will eventually be trained to earn their daily fluid intake. The animals will be carefully monitored for health and well-being. The neurophysiological recording and testing require that the animals' heads are restrained to remain still, so the animals will also be gradually accustomed to achieve this. Surgery will be required to insert recording devices and these devices will be placed based on data collected using magnetic resonance imaging (MRI). Similar devices have been used in different brain areas of human clinical patients and the standards of surgery and care are closely similar to

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

human clinical standards. The research programme is at the *Severe* level of severity as defined in DIRECTIVE 2010/63/EU.
This research project can only be carried out through neurophysiological recordings in awake behaving macaque monkeys, because this is the only available research method for directly linking the activity in single cells to behavioural performance in perceptual tasks. Particularly for this research, the stereoscopic vision and binocular eye-movements are similar to those of humans. Non-invasive human techniques, e.g. fMRI or MEG, are currently neither temporally nor spatially precise by themselves to study neuronal mechanisms.
Our experimental unit is not the individual animal, but the population responses of individual neurons; therefore, the number of animals required is small. For each identified part of the brain to be studied, results will be typically collected from two animals. A third animal is needed if findings in two animals are inconclusive. This project will examine three different brain areas. Additional animals may be needed if an animal cannot be trained in the specified time or if neurophysiological cannot be completed in an animal for welfare reasons. In these cases, although the data collected will be useful, our objectives will require additional animals. All behavioural experiments are based on the same perceptual task. Therefore, by carefully staging different parts of the protocol, all objectives can potentially be achieved with as few as 8 animals.
There are currently no other, non-invasive methods available to elucidate neuronal mechanisms and functional circuitry at the level of single neurons in real-time. In order to characterize a brain cell and relate its firing statistically to visual stimulus and behaviour requires many trials. The cognitive tasks are subtle and sophisticated, involving the testing of binocular performance, thus requiring the use of macaque monkeys. Animals will be carefully assessed for their suitability and will be socially housed. They carry out these tasks for fluid rewards in the context of restricted access to fluid at other times. Training schedules and rewards are tailored to the individual animal. The risks associated with general anaesthesia and surgery for skull implants are similar to those for humans; we work to the same aseptic standards. Animals will be regularly monitored for well-being by researchers and veterinary staff.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Pathogenesis of murine osteoarthritis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Osteoarthritis, cartilage, disability, joint replacement, pain</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[2]</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[3]</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">Osteoarthritis (OA) is the most common form of arthritis yet we do not have any treatments that are able to arrest or prevent disease. At the present time treatment is by pain relief and ultimately surgical joint replacement. For some forms of OA such as that affecting the hands, there are no effective therapies at all.
OA is characterised by loss of the articular cartilage, the smooth tissue at the ends of bone that allows frictionless movement of the joint. When this is eroded, many changes occur in the joint including remodelling of the bone and inflammation of the lining of the joint. These are thought to give rise to pain and stiffness that is characteristically experienced by the majority of individuals with disease. Despite it being a very common disease, we still have little understanding of the processes and molecules that are important in driving it and whether or not adult cartilage is capable of repairing itself once damaged.
The objectives of this programme are fourfold: (i) to identify the molecules that are involved in initiating the process of joint disease in order to find new treatment targets (ii) to understand the processes involved in repair of cartilage after it has been damaged so that we might be able to promote self-repair after OA has become established (iii) to understand how joint damage in OA leads to pain, to help design new strategies for managing the symptoms of disease (iv) to identify markers for disease in the blood and other body fluids so that it is possible to predict which patients will get disease</td></tr>
</table>

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

	and to monitor improvements in disease once we have novel drugs to test.
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)?	Work already published has demonstrated that it is possible to identify new targets for OA from animal models of disease. Some of these are currently being explored by the pharmaceutical industry. It is likely that strategies will be broadly divided into three groups: (i) therapies that prevent disease – this may be most appropriate for individuals in whom an initiating event in their disease can be identified e.g. acute joint injury; (ii) therapies that arrest or reverse established disease. Of the latter these are likely to involve promoting self-repair or blocking the pathways that drive on-going disease; (iii) therapies aimed at improving the symptoms of OA, which will include novel pain killers and blocking the processes that activate pain pathways in damaged tissues. As OA is a massive burden to society (through days off work due to back pain as well as the expense of performing joint replacement surgery), any improvements in the treatment of this condition will be of high relevance and substantial benefit.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice including genetically modified. Approximately 5650 mice will be used over a 5 year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	OA is induced in mice by a small surgical operation under general anaesthetic. This causes instability of the joint and is akin to the type of injury sustained by a footballer or skier following a twisting accident ("torn cartilage"). The mice tolerate this procedure well and despite having some pain immediately following surgery (lasting about 5 days) are fully active and apparently pain free up until about 11 weeks following the operation. At this stage only do they experience pain. Most experiments are terminated before this stage (except when pain is being examined specifically). Most animals (85%) are killed within 8 weeks of surgery and all within 20 weeks.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our lab has always used a combination of in vitro experimental systems alongside animal studies, but there is no substitute for being able to explore the complex processes that occur in the joint in the live animal. It is also the case that we validate all our mouse findings in human disease samples to check that pathways important in the mouse upon disease induction are relevant to the human condition.
2. Reduction Explain how you will assure	We have significant experience of this model and know that we can use relatively small numbers (8-

the use of minimum numbers of animals	10) in each experimental group to detect differences between groups. We ensure that some variables are always kept the same such as gender (male) and strain of mouse, as well as housing variables such as number of mice in a cage. We have a designated technician who manages the mouse colonies assiduously so that we only breed mice that are needed for designated studies.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The surgical model of OA that we use is extremely well tolerated and produces a very robust, though insidious, disease (like the human condition). This model is scored largely by examining histological sections of joints after the mice have been killed but we are developing methods that are more quantitative and which should help to shorten experiments and possibly reduce numbers. As we understand the pathways that are driving painful disease we are able to focus most of our efforts on earlier processes, which occur prior to the development of pain.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Targeting angiogenesis to improve stem cell transplantation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Stem cells, transplantation, cancer</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[4]</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[5]</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 remains a significant worldwide health problem, resulting in the death of more than 6 million people per year (http://www.who.int/en/). Stem cell transplantation is a widely established treatment for both leukemia and other blood diseases and disorders. In order to prepare a patient for a transplant, the disease is first treated and then the bone marrow prepared to receive the stem cells by irradiation and/or chemotherapeutic agent, in a process called pre-conditioning, stem cells are then administered to restore the blood system.

Currently, stem cells for transplantation may be sourced from umbilical cord blood (UCB), bone marrow (BM), or from mobilized peripheral blood from the patient or a donor. Approximately 70% of patients requiring a stem cell transplant have a suitable tissue matched family donor available. However, for some recipients, especially those from black and ethnic minority groups, a directed collection of cord blood following the birth of a sibling may currently be the only opportunity for a stem cell transplant. Despite the attraction of cord blood, compared to other stem sources, restoration of the blood system is often slow or fails. In addition, even transplants conducted with the patient’s own cells are not always successful. Slow</td></tr>
</table>

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

	or failed stem cell engraftment is a significant problem for the patient, as it is associated with increased morbidity due to the development of infection and extended hospital stays. Also, if the stem cells fail to restore the immune system death will occur. Successful stem cell transplantation is a multi-step process dependent upon the stem cells successfully reaching the bone marrow and the settlement of these cells into the tissue where they produce all of the new blood cells. In the adult, the bone marrow houses cellular components that form a specialised microenvironment or niche, tightly controlling the way the stem cell respond and form the blood system. Recent studies in mice have clearly demonstrated that pre-conditioning regimes prior to bone marrow transplantation can significantly affect the bone marrow vessels and that the a repair of the bone marrow vessel is essential in order for the stem cells to begin to restore the blood system. Leukaemia is a malignant disease arising in blood stem cells. Research has also supported that in the same way the bone marrow vessels supports the growth of healthy stem cells it can also provide protection and promote the growth of diseased ones. We therefore aim to test if we can improve the speed of blood cell recovery following stem cell transplantation and/or reduce disease burden or relapse of leukaemia using agents targeted to angiogenesis in bone marrow.
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)?	More than 50,000 stem cell transplants per year are performed worldwide. Side effects from delays in haematological engraftment or residual persisting disease itself (leukaemia) can result in significant suffering though complications such as infection, extended hospital stays and even fatality to the patient. We believe the potential benefits of our work will include: 1. Better scientific understanding of how normal and malignant stem cells grow in the bone marrow, this will facilitate the development of new treatments and avoid the need for repeating transplants, thereby improving patient lives and reducing NHS spending.

	2. By improving stem cell sources such as cord blood we widen the choice of material available for therapy, thereby impacting patient treatment and offering a better chance of tissue matched products for patient groups who would otherwise have difficulty in finding a match. 3. Refinement of existing stem cell therapies and facilitating the application of newer ones could mean shorter hospital stays for bone marrow transplant recipients as time to haematological reconstitution shortens. This would improve patient lives and reduce spending in the National Health Service, leading to more resources available to other patient groups.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use a maximum of 2500 mice over the 5 year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The majority of the genetically modified strains of mice used in this study will have no apparent defects compared to wild type animals and will therefore not exceed a mild severity level. However, it is possible that some animals used in this project could exhibit symptoms such as bone defects or slow healing after injury due to their genes, these animals are expected to be infrequent and will not exceed a moderate severity level. Irradiation may lead to symptoms such as weight loss and infection as they do in humans, this does not happen in all animals and usually only occurs for a few days. Animals showing adverse effects due to irradiation will not be permitted to exceed a moderate severity level and any animal showing significant signs of suffering and distress will be culled immediately.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have and will continue to screen intensively in the laboratory without the use of animals where possible, but we cannot screen our test compounds in the stem cell transplantation process without the use of animals as this can only be modelled in the whole animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will only test agents that have already proved effectiveness in tests performed *in vitro*. Experiments will be carefully planned and conducted to ensure that only the minimum number of animals necessary will be used.
3. Refinement Explain the choice of species and why the animal model(s)	Mice are a species with the lowest neurophysiologic sensitivity that we can use to produce scientifically

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.	valid results that closely model the stem cell transplantation process that occurs in humans. Irradiation effects will be minimised by using the lowest dose necessary and by administering the dose over two times. Alternatively, chemotherapy may be better tolerated and in these occasions this will be used. Infections will be prevented by administration or prophylactic antibiotics. Animals administered with leukemic cells and animals treated with these cells will be monitored carefully for signs of excessive disease burden, such as excessive weight loss or high levels of leukemic cells in the system circulation, or disease spread to other sites. Our monitoring procedures will mean that any signs of suffering or distress will be picked up quickly and any necessary action taken to minimise welfare costs to the animals.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Develop antibody drugs for cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cancer, drug, antibody, research</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[6]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Regulatory use and routine production</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Preservation of species</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Higher education or training</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[7]</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>Antibodies are a new class of drugs that can better recognise and kill cancer cells in cancer patients. There are a few antibodies drugs being used in treating breast cancer, leukaemia, and colorectal cancer, and they are all very effective. More of such drugs can be developed to treat other cancers and to help cancer diagnosis or research.</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>We propose to produce one or two antibody drugs for cancer treatment at the end of the programme. These antibodies will have been tested in test tubes as well as in animals for their ability to recognise and kill cancer. They can then be ready for clinical trials to be tested in humans. Not all the antibodies produced will be suitable for cancer treatment: some of them will be used for diagnosis; some may help doctors decide which other drugs are more suitable for certain patients; and other antibodies may be useful in basic research.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>We plan to use mouse only, and we expect to use 1350 mice over the 5-year period.</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 will be injected with drugs to help them produce antibodies. Other animals will be injected with cancer cells for them to grow tumours, and then drugs will be used on them to test the effects of treatments on tumours. There will be discomfort after injection; some may be at the level of moderate severity. The animals will develop tumours that affect their general appearance and movement. Some tumours may cause skin ulcer.</td></tr>
</table>

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

	The animals will be monitored closely and those have grown oversized tumours will be sacrificed. All animals at the end of the experiments will be sacrificed humanely.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Mouse is by far the best model for producing antibodies. Alternative approaches are being developed, but none of them has become as reliable as the mouse model. We have tried one of the best alternatives, but could not make any antibody. Tumour growth is a complicated process involving many aspects from both the tumour and the host (human or animal). So far scientists have not been able replicate this process in test tubes. Testing drugs is more complicated because drug delivery is affected by the blood supply to the tumours, and by how deep the drugs can penetrate the tumour blocks. But new drugs can be tested in cell culture first so that ineffective drugs do not need to be further tested in animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In antibody production we make sure we use the best practice and technique to ensure the success of obtaining good antibodies, consequently avoid using more animals to repeat the experiment. In tumour growth and antibody treatment studies, we always screen the drugs in cell culture first so we do not have to test ineffective drugs in animals. We also speak to statisticians about experiment design so we do not use more animals than we need to. We also use special mice that have the same genetic background so that they will show similar responses to tumours and to drugs, which will give us a better idea of how effective the drugs are.
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.	Mouse is by far the best species for making antibodies and for studying tumour treatment in most cases. We have over the years found the best strains to use for antibody production, and will continue to do so. And we also give special drugs to the mice to help them produce better antibodies and to reduce the discomfort caused by the procedures. In tumour models we use the well-documented and well-tested strains to grow tumours and to test new drugs. In some studies, we test the drugs initially with small numbers of mice to have an idea of how effective they are before scaling up the experiments. During experiments, we make sure the animals are checked regularly, and signs of distress will be dealt with promptly.

Brain co-ordination and vital behaviours
Brain, hypothalamus, sleep, alertness, body weight

- Summarise your project (1-2 sentences)

Coordination between different brain regions, and the body, is necessary to avoid the most frequent medical problems of today, such as obesity and insomnia. Our aim is to understand brain mechanisms that achieve healthy body coordination and avoid obesity and insomnia.

- 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.

Recent work identified which brain cells are key to preventing obesity and insomnia, but it is not known how they prevent these diseases. This is what we want to find out, by studying how specific brain cells regulate sleep, perception, and body weight and how different conditions (eg. diets) affect vital brain cells. We aim to address this by a combination of state-of-the-art techniques (such as optogenetics and electrophysiology) that give very precise answers and so limit the number of animal experiments required to gain unambiguous knowledge about key medical problems such as obesity and insomnia.

- Outline the general project plan.

Breeding and phenotyping transgenic mice will be performed to determine which genes are important for health and behaviour and provide tissue for ex vivo studies of regulation of individual cells. In parallel, brain recording/control devices will reveal what type of neural activity controls health and behaviour, and how neurons influence each other.

- 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.

In some experiments, animals will undergo behavioural tests (e.g. measurement of their locomotor activity) that cause little or no stress. In others, minor surgery will be performed with appropriate anaesthesia/analgesia, from which the animals are expected to fully recover. We will work closely with local vets to optimise our experiment for minimal animal suffering.

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

Overall, our work is expected to provide novel information about the operation of brain-body coordinator networks. It will advance our knowledge of how different brain and body system function together for successful perception and behaviour. It will also provide new guidelines for treatment of key disorders of brain-body coordination such as obesity and insomnia, that now affect around 1 in 4 people in the Western world.

This work will also increase our basic knowledge of how neural systems signal to one another.
The new tools developed in this project will be valuable to other scientists interested in neural signalling, brain-body communication, and brain coordination.

- 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.

For the experiments necessitating animal use, in this long-lasting major research programme we will use mice or rats (6 for a typical set of experiments). Mice will be used because they are species with lowest neurophysiological sensitivity that are still close enough to humans, and rats will be used in some experiments because the mouse skull is too small for some experiments (e.g mounting a wireless brain probe). All experiments will be subject to prospective and retrospective statistical and ethical review to ensure the appropriate number of animals are used throughout the lifespan of this project.

- 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.

We have to use animals (mice and rats) because we would like to understand the biological bases of human health and disease. These animals are sufficiently close to humans to draw important conclusions relevant for human therapies, while it is unethical to experiment on humans directly for the type of mechanistic studies that are necessary. We will refine our procedures by subjecting our research staff to rigorous training and by constant consultation with vets. In parallel, where possible, we will build and use computer simulations of, for example, neural networks, to complement and reduce animal use.

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

We will follow the latest government and veterinary clinical guidelines on animal procedures. Animals will be maintained in climate controlled rooms with ample food, social interactions, and environmental enrichment. Latest equipment and methods (e.g. anaesthetic machines, microsurgery kits, aspectic procedures) will be employed in all procedures. Animals will be closely monitored for signs of distress and procedures stopped immediately if any severe suffering is suspected. Compared to government-approved programmes that account for the majority of animal use in the world today (i.e. farming), our research programme programme will involve similar or smaller discomfort to animals, and vastly smaller numbers of animals. Furthermore, the results of our research may well benefit animal, as well as human health, thereby further reducing net animal suffering.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Regulation of Immune Response in Rodents</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">immunoregulation; infection; inflammation.</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">Five</td></tr>
<tr><td>Purpose of the project (as in Article 5)[8]</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>
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<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[9]</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">Our aim is to study the various molecules and mechanisms which trigger the immune response to achieve long term protection from infectious agents, with minimum pathology, and the interaction between pathogens and their hosts that determine whether or not infection will persist.

Infectious diseases are a leading cause of death worldwide, and are increasing in almost every nation. They are also among the biggest disablers. Almost 90% of deaths from infectious diseases are caused by only a handful of diseases. Most of them have plagued mankind throughout history, often ravaging populations more effectively than wars (WHO Report on Infectious Diseases). Protection against infection by viruses, bacteria and other pathogens as well as the efficacy of vaccination crucially depend on appropriate activation of the immune system, a complex and vital network of cells and organs that fights invading pathogens. An understanding of these molecular pathways is essential for the design of vaccines for prevention and intervention in viral and bacterial infections.</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 results of the research carried out under this project will be disseminated through publication in high quality peer reviewed journals and at meetings. The potential benefit is an improved understanding of the immune response to persistent pathogens, such as those causing TB and AIDS. The knowledge gained from the vaccine experiments may be directly transferable to human vaccine studies or trials either through the MRC or</td></tr>
</table>

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

	by other interested parties. The studies on immunopathology may lead to a better understanding of the molecules and cells contributing to pathogenesis. This knowledge may help in the design of intervention therapies in clinical therapeutics in immune pathologies.
What species and approximate numbers of animals do you expect to use over what period of time?	To achieve the objectives outlined above we propose to use the laboratory mouse as the model organism. We estimate that we will be using approximately 30,000 mice per year, the majority of which (~70%) will only be used for breeding.
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?	An essential feature of the immune response to an infectious microorganism is that it is enhanced by a second exposure (i.e. has memory) and this quality forms the basis for vaccination. However, despite triggering activation of the immune system, many viral and bacterial pathogens are able to establish a chronic infection, often associated with significant disease burden (e.g. *Mycobacterium tuberculosis* and HIV-1). Moreover, excessive or inappropriate activation of the immune system may result in inflammatory immunopathology (e.g. in influenza infection and other infections or inflammatory diseases), which is normally regulated by a number of suppressive cell-types and mediators. This application is made with the primary purpose of understanding the complex interaction between pathogens and their hosts and the mechanisms that regulate the immune response, leading to protection from pathogens with minimum immunopathology. We are proposing to use appropriate mouse models for clinically-relevant infections, in which the parameters of protective immunity can be studies and tested. The proposed programme of research is to provide the basic immunological foundation upon which rational vaccine design and inhibition of immunopathology during chronic inflammation and/or autoimmunity can be based. Mouse models for infection with *Mycobacterium tuberculosis*, retroviruses or influenza A viruses will be studied in detail. In addition, genetic alteration of specific genes will reveal their precise role in the response infection, autoimmunity or cancer. Common to all infectious models is the assessment of virulence or pathogenicity. This will be assessed most frequently as morbidity, which is quantifying changes in physiological parameters relevant to each type of infection and clinical symptoms associated with all types of infection. Many of the pathogens proposed to be used in the project can

	cause disease. Every effort will be made to prevent infection-associated pathology.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Initially, the role of different mediators and compounds, which can affect the induction of an immune response to and/or growth of a pathogen, will be determined in *in vitro* studies. Ultimately, however, the immunological and immunopathological investigations, and particularly vaccine and antimicrobial effectiveness and autoimmune pathologies, cannot be carried out without the use of animals, since the host's immune system cannot be entirely mimicked by any *in vitro* assay. Furthermore, although all compounds will be selected for *in vivo* testing based on evidence of activity in relevant *in vitro* assays, this cannot replace the *in vivo* tests under the physiological conditions of an infection, as potent *in vitro* activity might not translate into an *in vivo* activity.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The design of quantitative experiments will be based on implementation of Reporting of In Vivo Experiments (ARRIVE) guidelines published by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3R) (PLoS Biol 8(6): e1000412. doi:10.1371/journal.pbio.1000412). These guidelines will allow us to calculate with accuracy the minimum number of mice required to obtain a scientifically meaningful result. Using too few mice would lead to inconclusive results. The use of dedicated computer databases for mouse breeding and management has been implemented at NIMR. This allows us to carefully monitor the mouse breeding programme and also share mice with selected genetic traits between investigators so that duplication is avoided. Cryopreservation of gametes, embryos, tissues and cells is also routine at NIMR and will ensure that the minimum number of mice is bred.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The mouse is the best characterised model for these studies, with many features applicable to human infection. Their immune responses are well defined and the technology enabling sophisticated manipulations of the haematopoietic and immune system is highly developed. Mouse transgenic and knockout techniques are well established; mice have a relatively short generation time; its haematopoietic system has been extensively studied and, in addition to the accumulated knowledge, there exists a vast array of reagents

	that facilitate the studies to a level unknown for many other organisms. To our knowledge no other species of lesser sentience can fulfil the requirements of this project to the same extent as the mouse. In our experiments, specific end-points will be used to monitor the effect of infection on physiology, instead of induction of pathology, and we will go to great lengths to minimise the possibility of severe pathology. This will be achieved by close monitoring of symptoms and physiological parameters during the course of the infection. We will use genetically-modified micro-organisms as challenge strains in order to take advantage of reporter signals – luminescence or fluorescence, for example – to monitor the course of infection and potential dissemination to different organs with non-invasive techniques.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Development & homeostasis of the vertebrate nervous system</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Central, peripheral, enteric nervous system, embryogenesis, signalling</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)[10]</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[11]</td><td>Yes</td><td></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The work described in this Project licence aims at defining the molecular and cellular mechanisms by which the highly complex nervous system of animals is built during embryogenesis and how it is maintained in a healthy state during adult life.

The nervous system is responsible for receiving and processing information relating to the state of the outside environment, has a critical role in the planning and execution of movements and is the site of higher intellectual activity. In addition, the nervous system has an important role in growth as well as digestive and metabolic functions.

The overall aim of this research program is to identify pathways associated with the development and function of the nervous system, in normal and pathological conditions.

Specifically, our work has two objectives:
1. To define molecular and genetic pathways which regulate development and homeostasis in the nervous system along the gut-brain axis.
2 . To identify conditions which influence the function of the nervous system in normal and pathological conditions.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or</td><td>One of the main interests of our research is to understand how genetic changes lead to abnormal pathophysiology in humans. Such studies provide crucial information regarding the pathogenesis of</td></tr>
</table>

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

animals could benefit from the project)?	disease conditions and are instrumental for the development of novel therapeutic strategies. We will also be adding to the pool of scientific knowledge and will make all animal models, reagents and information available to the science community.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice and zebrafish are the only species used in this project. Over the lifetime of the project, we may use a large range of genetically distinct lines. We have requested authority for 50,000 mice and 6,000 fish but this includes a large number of genetically normal animals, post genotyping, that will not undergo further analysis.
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?	Prior to animal work, we carry out biochemical and cell culture experiments which, in combination with information obtained from public access databases, identify candidate genes that are essential for the formation and function of the nervous system. This information is then used to generate transgenic animals in which the expression of selected genes is modified. Our main experimental approach involves histological and microscopic analysis of post-mortem tissues and physiological analysis of organs isolated from transgenic animals. Therefore, in the majority of cases, there will be no further interventions other than those required for breeding and genotyping. However, to reveal the phenotype in those cases where the genetic alteration is conditional, we will administer substances such as tamoxifen or doxycycline that induce/repress/modify gene expression. These will be administered by the most appropriate route, selected for the minimal invasiveness compatible with efficient delivery to the target tissue. In less than 10% of cases we may do further analysis of phenotype by behavioural observation or imaging. In other cases we will do in utero manipulation of developing foetuses to investigate the role of stems cells. In a very small proportion of animals, <1% we may follow specific pathways of epilepsy. For the large majority of this work the only procedures will be those involved in breeding and maintenance of genetically altered lines of mice and zebrafish. It is expected that all but a few animals will experience no more than mild adverse effects.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To define the molecular mechanisms by which genes and cells control the development and function of the nervous system that are relevant in the context of the whole intact animal and are

	therefore meaningful for human clinical studies, it is necessary to utilize animal experiments. In particular, the effect of different genes and proteins and their interaction with each other is also influenced by nutrients, oxygen, circulating hormones and other aspects of the complex physiological environment inside the body. It is not yet possible to recapitulate all of these parameters in vitro, nor to mimic the metabolic crosstalk between different populations of the ENS and gut-brain axis – a key aspect of this research project. In addition, the effects of manipulations of the diet can only be meaningfully studied in vivo. Nevertheless, our current and future research makes extensive use of alternatives to animals. In vitro cultures of mammalian cells/tissues can be used for many of our studies and are undoubtedly an important source of replacement. Before embarking on any animal experiments, we will collect as much evidence as possible to determine whether a candidate genetic or environmental manipulation has a reasonable chance of success and providing information within *in vivo* systems. Evidence will be collected from our own experiences and previous results as well as by surveying the mammalian and other literature. In addition, we will use non-regulated procedures to collect expression data from fixed non-GM mammalian tissues and functional/expression data from genetically and/or environmentally manipulated *in vitro* mammalian cell lines and/or early chick and fish embryos.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Where possible, GA lines will be maintained in a homozygous state, thereby obviating the generation of a large excess offspring with inappropriate genotypes. In other cases, homozygotes will be generated from heterozygote intercrosses, with littermates genotyped as heterozygous or wild type used as age and gender matched controls. Whilst most (~80-90%) of the experimental work will be *ex vivo* following breeding where any physiological or other interventions are required, we expect that 5-6 animals per treatment group will usually be sufficient to obtain robust results. For most of the quantitative experiments, design will be based on ARRIVE guidelines and sample sizes may be set using power analysis, generally using a significance level of 5%, a power of 80%, and a least practicable difference between groups of 20%. Otherwise, we will use the minimum number of animals to provide an adequate description, generally on the basis of previous experience (our own, or from the

	literature). This programme of work will make optimal use of several tissues, fluids and cell types per individual mouse. We will aim to collect organ samples from multiple body sites and to provide other affected tissues to appropriate scientists, so that they do not have to breed mice specifically for their experiments. This highly integrative approach will maximise the information obtained from the minimum resources. Cryopreservation of gametes, embryos, tissues and cells is routine and will ensure that the minimum number of mice is bred.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We are using refined GA mouse and zebrafish models, employing conditional and inducible technology where appropriate. To minimise stress during breeding and maintenance, we will follow best practice guidelines and follow local refinements of husbandry such as cage enrichment and sufficient amounts of nesting material. On receipt or generation of a new line, we will minimize suffering by ensuring increased observation and monitoring until a detailed phenotypic analysis for each line is accomplished. If any welfare implications are identified, they will be acted upon and refinements considered in consultation with the NVS and NACWO. The majority (~95%) of animals produced under the breeding protocol are not expected to exhibit phenotypes beyond a mild classification but a small proportion may exhibit a moderate phenotype - particularly if they are modelling a human disease. However, it is not possible in all cases (such as newly generated lines) to predict fully the nature or severity of any potential defect and for that reason the limit has been set at moderate. For all types of mice, however, there will be careful monitoring of strain characteristics and the information will be collated and regularly reviewed to ensure that phenotypes to not exceed their usual features. For all manipulations we will adhere to local or national guidelines that aim to minimize suffering. Most of the work as well as the administrations of gene inducers/repressors or other agents are standard and previous refinements from our own experience and from the literature will be used. If, however, there is insufficient information available, new manipulations will be pre-screened in small-scale pilot studies to obtain indications of the minimum dose and exposure time that is likely to be effective, thereby minimising any potential suffering.

	Unless otherwise specified, all surgical work in this project will be undertaken in accordance with the principles set out in the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2010) or other such publication promoting best practice. Analgesia will be provided according to contemporary best practice and advice from the NVS/NACWO.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Patterning embryos with genetic oscillations</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Embryology, scoliosis, biological clocks</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)[12]</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[13]</td><td>Yes</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The segmentation clock is a newly discovered embryonic patterning system that regulates the sequential and rhythmic formation of the precursors to the skeleton of the vertebrate embryo. It consists of a population of oscillating, coordinated cells in the out-growing tip of the embryo. With each tick, the pattern of the next vertebral body is established. When it fails, the embryo makes a defective axial skeleton, which in humans is termed congenital scoliosis. The patterning of tissue by genetic oscillations is a novel paradigm, and the molecules and mechanisms remain largely mysterious.</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>Our experiments will determine what regulates the ticking of this segmentation clock, and how this timing is translated into the anatomy of the adult. Knowledge of the zebrafish segmentation clock will be shared with our clinical colleagues, and may contribute to better diagnosis or treatment of human congenital scoliosis.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>We will use 100,000 adult zebrafish over the 5 year duration of the project. Their role in the project is to provide embryos for experiments at non-regulated stages.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</td><td>The procedures applied to regulated stages of zebrafish are used for characterizing and maintaining lines, and are all well established of a mild severity. There are no expected adverse effects.</td></tr>
</table>

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

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The segmentation clock is a complex, multicellular embryonic process that currently can only be studied in vivo. We are developing a primary cell culture model with which certain questions might be tractable in vitro. We use mathematical simulations of the system to analyse and refine the experimental questions.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our study design reduces the total number of animals by using (1) live reporter lines, allowing time series data to be collected from single animals; (2) homozygous viable mutants, obviating the need to maintain carrier lines; (3) mathematical simulations, focusing the type and reducing the number of experiments; (4) sharing lines, where possible, with other researchers.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We use the zebrafish because (1) its anatomy and genetics are a good model for other vertebrate species; (2) its embryos are externally fertilized and can be obtained without harm to the mother; (3) they are near transparent, facilitating imaging studies. All zebrafish will be maintained according to best practice. Short-term anaesthesia and analgesia will be used where necessary.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Molecular switches in inflammation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Arthritis, Asthma, Macrophage, Inflammation, Molecular switch.</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)[14]</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[15]</td><td>Yes</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The consequences of severe inflammatory conditions include a wide variety of diseases with huge social impact ranging from autoimmune diseases to asthma, heart conditions, Alzheimer’s and cancer. We are interested in a specific type of immune cell which plays a key role in inflammation and are essential components of our defence against these diseases. My laboratory has recently discovered the identity of the ‘master switch’ of these immune cells and we believe that, by turning this switch on or off, we can dampen down inflammation present during autoimmune disease or, alternatively, boost the immune system in people with a compromised defence against disease. Our strategy is to understand how this master switch of macrophages can be controlled at the molecular level by interfering with its function and thereby, develop new ways of reducing inappropriately sustained inflammatory responses.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>This work has the potential to result in new therapies against potentially debilitating inflammatory disease, such as asthma, arthritis and some heart diseases. Our previous studies have made considerable contributions to the understanding of the inflammatory response and our continued work will lead to a new understanding of these diseases, which will significantly contribute to new approaches to diagnosis, prevention and treatment.</td></tr>
<tr><td>What species and approximate numbers of</td><td>Mice, approx 8,200 over 5 years.</td></tr>
</table>

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

animals do you expect to use over what period of time?	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	All experimental procedures are classed as mild or moderate. However, there is the potential for adverse effects to occur. Our work can be divided into two main areas and each area is associated with the potential welfare issues; 1. Breeding and maintenance of genetically modified animals. We do not expect any harmful side effects purely from breeding genetically altered animals; however the animals will be monitored daily and should any unexpected side effects occur the animals will be immediately euthanized by a pre-approved method. Where the immune status of the animals might compromise health, the animals will be maintained in a barrier environment, which will protect against infection. 2. Procedure designed to model human disease by administration of disease modifying substances. Any animal which undergoes any procedure which has the potential to elicit suffering will be monitored for signs of possible side effects and animals exhibiting signs of distress exceeding the humane end-points (any mouse showing any two of the following: dyspnoea, ruffled fur, weakness, dehydration or a hunched appearance, or has lost 20% or more of its body weight) will be immediately euthanized. Anaesthetics and pain-killers will be routinely provided to all animals when required. In models where analgesics cannot be given because their anti-inflammatory activities tend to inhibit the induction and progression of the disease (e.g. arthritis), additional veterinary support such as easier access to food and water (e.g. food pellets and water gel packs placed at floor level), and/or supplemental bedding may be provided during advanced stages of disease. Surgical procedures will adhere to strict protocols involving appropriate anaesthesia and administration of pain killers, so as to minimise any suffering. We will employ strict, pre-defined criteria in order to assess whether an animal is in distress and animals undergoing procedures will be monitored daily and will be euthanized using pre-approved methods should they show signs of ill-health. Importantly, we will never work outside our pre-approved animal licence, which details the

	procedures which we may perform and sets strict rules and standards for the treatment of the animals under our care.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our previous work on the role of IRF5 in macrophage polarization was chiefly conducted in human cells. We now seek to understand how this regulator works in inflammatory diseases, but the steps leading to the development of the state of chronic inflammation are poorly understood and it is likely that multiple physiological processes are involved. This inevitably involves the use of whole organisms and, in particular, the use of animal models of inflammation. Our experiments will aim to duplicate as closely as possible, the symptoms experience by those suffering from inflammatory diseases; we will do this by administering animals with substances which induce the relevant changes the immune system. In addition, we will use genetically modified mice, lacking this regulator, to directly address its function during chronic inflammation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	As much as possible, we will complement the information generated from live animal models using isolated human cells, cell lines or tissues taken from patients with informed consent. We will use the minimal number of animals to achieve 90% power to detect difference between mean clinical scores with a significance level of 5%. We will base our calculations on previous experiments with a help of a biostatistician. Whenever possible, untreated control groups will be shared between treatment groups.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice and rats are the lowest vertebrate groups on which well-established models of inflammatory diseases of interest have been developed. Mice are preferable to rats because of the greater availability of reagents (e.g. monoclonal antibodies) specific for this species. The specified models have already been refined to minimise the number of immunisations and the severity level of adjuvant. Animal welfare is of critical importance during these experiments and is essential to obtain high quality data; therefore we work closely with animal housing staff and veterinary surgeons to ensure the highest standards of maintenance and welfare for mice under our care. Anaesthetics and pain-killers will be routinely provided to all animals when required. In models where analgesics cannot be given because their anti-inflammatory activities tend to inhibit the induction and progression of the disease (e.g. arthritis), additional veterinary support such as

	easier access to food and water, and/or supplemental bedding may be provided during advanced stages of disease. The most humane practice, however, is to limit the length of the disease to the shortest possible time required to answer a given experimental question. Occasionally, longer experiments will be carried out but in all cases, mice which have exceeded the humane end-points (any mouse showing any two of the following: dyspnoea, ruffled fur, weakness, dehydration or a hunched appearance, or has lost 20% or more of its body weight) will be culled.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Production of antibodies: DNA replication research</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Antibodies, Antigen, Immunisation</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)[16]</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[17]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The purpose of the project is to produce monoclonal antibodies that can be used to identify proteins in cells or in extracts prepared from tissues and cultured cells.
In our studies on the cell cycle we require antibodies to identify the proteins that are involved in DNA replication. Animals previously injected with known antigens will produce antibodies and these antibodies are very effective markers to help us identify the proteins involved in DNA replication
The enzymes required for DNA synthesis have been characterised and a lot is known on the enzymology but we are now interested in how different protein complexes are formed to enable the cell to both synthesise new DNA and subsequently duplicate their chromosomes to produce daughter cells. We would also like to know the identity of the proteins that are involved in DNA repair. The cell is continually monitoring the integrity of it’s DNA ensuring that aberrant DNA is corrected. Which of the many repair proteins are involved and where exactly they interact with the DNA replication are other aspects we are interested in. This has implication on the progression of cancer in many cells. Using these antibodies we can identify the proteins involved in DNA replication and repair.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the</td><td>The antibodies that we will raise from these mice will be of enormous benefit in the study of DNA replication. They will be used to identify DNA replication proteins and those involved in DNA repair. This will not only increase our knowledge of</td></tr>
</table>

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

project)?	DNA replication but will enable us to understand how these proteins are involved in progress of human disease. Increasingly we are finding that these antibodies are valuable tools in the diagnosis of human disease. Since we will have a limitless supply of the antibodies we will not have to rely on further immunization to replenish our stocks and these antibodies will be available to others as a constant supply should they be required by the scientific community.
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 as they are the most appropriate animals to use. In total we expect to use 180 mice per year and 900 mice over the 5 year term of the project.
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 injected with known proteins and after a period of time blood samples will be taken. The serum is tested and mice that have responded will be humanely killed and the spleens removed. These antibody producing spleen cells will be fused with standard tissue culture cells and the resulting hybridoma will be used as a constant source of antibody. During the immunisation period mice will not suffer any effects from the injected proteins. However on some occasions we have found that individual mice may suffer an immunological response after the final injection resulting in anaphylactic shock. This is very unusual and we take all precautions necessary (for example reducing the dose of antigen) to ensure that this does not happen. We monitor the mice regularly and frequently. On any very rare occasion that a mouse appears to be in distress we will kill the mouse immediately. Following an immunisation schedule, an individual mouse (the best responder) is removed from the group for use in an *in vitro* fusion procedure. When the fusion has proved successful, the remaining animals are killed at the same time to prevent stress caused by isolation.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animals produce antibodies with the highest affinity. New technologies are available which produce antibodies in-vitro, however, these are low affinity antibodies and although we have tried to use them in our work, none have been successful. This is partly due to the low abundance of the proteins that

	we are trying to detect. To be able to locate these low abundant proteins we need antibodies of very high affinity. The animal response so far is the only process that will generate these high affinity antibodies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	If an antibody is required, other sources will be exhausted before any animals are immunized. The number of animals that will be required is determined by considering the probability of the animals responding to the required antigen. Past experience on how mice have responded will be evaluated and taken into consideration as to how many mice are needed per group. Standardised immunization schedules with proven success will be followed and an adequate number of animals will be used to achieve appropriate response so that we hope to keep the animal usage to a minimum and thus avoid repeat immunizations and subsequent further need for more mice.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We use mice because they respond well to antigen injection and the donor cells used to fuse to the spleen cells are of mouse origin. Using cells of similar origin gives the best chance of success. We immunize the lowest number of mice necessary to ensure that at least one will respond sufficiently well to enable us to proceed with the experiment with a good chance of success in producing an antibody. We have reduced the number of injections and the volumes to be injected to the minimum. We have the reduced the number of test bleeds and now only take samples after the third and fifth injections. In nearly all cases the response after the fifth injection is stronger than after the third. We have found that further injections do not enhance the response so we have concluded that about five injections will produce a response that is sufficient. Adjuvants are only used when they are essential to generate an adequate immunological response. Only the smallest blood sample required will be taken for serum analysis. We will minimise suffering to the mice by regular and frequent monitoring and immediately killing any mice that are unexpectedly ill or showing signs of poor health. The health and welfare of the animals will be maintained by dedicated professional animal technologists and care staff. Animals are housed according to legal guidelines on temperature,

	humidity, lighting and noise levels. Wherever possible, we will strive to go beyond minimum housing guidelines, for example by providing refuges, nesting material and chew blocks. Animals are housed together at a young age usually in groups of 4-5 of the same sex per cage and they remain together until the procedure has completed.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Optimizing pharmacotherapy based on PK principles</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Pharmacokinetics, Pharmacodynamics, Lymphatic transport, Biodistribution</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)[18]</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[19]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>1. To elucidate the contribution of intestinal lymphatic transport to immunomodulatory activity of orally administered lipophilic cannabinoids. The project is important in improvement of treatment of multiple sclerosis.

2. To assess the pharmacokinetics and oral bioavailability of novel highly selective cyclin-dependent kinase 9 (CDK9) inhibitors for treatment of chronic lymphocytic leukemia (CLL). The project is important for improvement of treatment of CLL patients</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><u>Elucidation of the contribution of intestinal lymphatic transport to immunomodulatory activity of orally administered lipophilic cannabinoids.</u>
The proposed research programme will provide knowledge about the role of the intestinal lymphatic system in the absorption pathway of cannabinoids after oral administration.
The degree of immunomodulation will provide information about the feasibility of the lymphatic targeting approach of cannabinoids for the improvement of the treatment outcomes of multiple sclerosis.
With help of the proposed research project, a decision about a clinical study on multiple sclerosis patients will be made.

<u>Assessment of the pharmacokinetics and oral bioavailability of novel highly selective cyclin-dependent kinase 9 (CDK9) inhibitors.</u></td></tr>
</table>

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

	The proposed programme will provide information about the pharmacokinetic properties of the leading drug candidates. The candidates for the in vivo studies will be carefully selected based on selectivity and potency. Assessment of pharmacokinetic parameters in rats will provide a basis for a decision with what compounds to proceed in future work to efficacy studies on animal models of chronic lymphocytic leukemia.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 948 rats and 504 mice over the 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?	In a number of protocols a surgery under general anaesthesia will be used. The potential side effects include postoperative pain, bleeding and infection. The likely level of severity of these protocols is moderate. Other protocols involve minor procedures such as oral gavage or administration of substances by injections. These procedure will involve only mild discomfort and therefore will not likely exceed mild level of severity. The animals will be killed by schedule one method after the end of an experiment. Some of the animals that will undergo procedures and surgeries under general anaesthesia will not be allowed to recover from general anaesthesia and will be killed while on general anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The studies that aim to assess the pharmacokinetics of drugs require the interplay between a chemical compound and physiological functioning of different systems in the body. At this time, no *in vitro* or *in silico* methods exist that are able to totally replace animal experiments for generation of reliable information about pharmacokinetic behaviour of drugs in the body.
2. Reduction Explain how you will assure the use of minimum numbers of animals	**Objective 1:** <u>Measures to ensure that the minimum number of animals will be used in this project:</u> We will assure the use of minimum number of animals by performing two studies (one is in silico (computer-based) and one ex vivo (using lipoproteins derived from rat blood) before we perform studies in animals. This will assure that only compounds with high chances for intestinal lymphatic transport would reach the step of animal studies and therefore will lead to a smaller total number of animals used in these studies. **Objective 2:** <u>Measures to ensure that the minimum number of animals will be used in this project:</u>

	An extensive in vitro screening of the synthesized CDK9 inhibitors candidates will be performed before any in vivo experiments will be done. The screening will involve selectivity studies with kinases and potency studies using cancer cell lines, CLL cells as well as normal lymphocytes. Only 3 most selective and most potent compounds will proceed to in vivo studies.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	**Objective 1: To elucidate the contribution of intestinal lymphatic transport to immunomodulatory activity of orally administered lipophilic cannabinoids.** Choice of species: The ex-vivo model for association of drugs with chylomicrons was established using rat chylomicrons and therefore for the model to be predictive for the lymphatic transport potential it is logical to use chylomicrons derived from the same species. For pharmacokinetic assessment and lymphatic transport of cannabinoids we choose rat as a model. Rats are the most widely used model for pharmacokinetic assessment of drugs and lymphatic transport and there is significant amount of previous work that has been done with pharmacokinetics and lymphatic transport of drugs (including some cannabinoids) using rat model The size of the rat allows multiple blood sampling of relevant volumes from the same animal– which results in lower number of animals used versus mice. For biodistribution and immunomodulation assessment studies we chose to use both rats and mice. Rats will be used in order to compare the biodistribution results to the pharmacokinetics and lymphatic transport results. Immunomodulation assessment studies in rats will be performed in order to compare the immunomodulation to the pharmacokinetics and because there is significant amount of information about cannabinoid-induced immunomodulation using mouse lymphocytes **Objective 2: To assess the pharmacokinetics and oral bioavailability of novel highly selective cyclin-dependent kinase 9 (CDK9) inhibitors for treatment of chronic lymphocytic leukemia (CLL).** Choice of species: For pharmacokinetic assessment of CDK9 inhibitors we choose rat as a model. The size of the rat allows multiple blood sampling of relevant volumes from the same animal– which results in lower number of animals used versus mice (the entire pharmacokinetic profile of a given drug can be followed in individual animals).

	Measures to minimise welfare harms to the animals All procedures that can potentially lead to moderate severity limit (such as surgery) will be performed under general anaesthesia. In order to minimise postoperative pain we will make sure that appropriate pain relief medications are used.