Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 23

Projects with a primary purpose of:
Translational and Applied Research – Human Immune Disorders

Project Titles and keywords

1. **Development of hiv-1 vaccines**
    - HIV-1 vaccine, mice, immune responses

2. **Autophagy in the hematopoietic system**
    - Autophagy (self-eating), leukemia, autoimmunity, ageing immunity, vaccination

3. **Immunity, tolerance & treatments in type 1 diabetes**
    - Immunology, type 1 diabetes, infection, autoimmunity

4. **Immune regulation, autoimmunity and infection**
    - Autoimmunity Infection Disease Prognosis

5. **Evaluation of Antibody Therapy in Immune Models**
    - Monoclonal Antibody, Immune therapy, Inflammation

6. **Coagulation Proteins in inflammation and immunity**
    - Inflammation, thrombin, immunity, atheroma

7. **Mechanisms for Immunological Memory and Tolerance**
    - T cell memory, Regulatory T cell, Autoimmunity, Allergy

8. **Macrophage biology in rodents and livestock**
    - Macrophage, Csf1, Csf1r

9. **Development of the neonatal immune system and links with disease**
    - Immune system, neonatal, disease

10. **Antibodies for research and clinical purposes**
    - Monoclonal antibodies

11. **Production of Antibodies**
    - Monoclonal, polyclonal anti-sera

12. **Service monoclonal and polyclonal antibody production for Biological & Biomedical Research**
    - Polyclonal and monoclonal antibody production

13. **Immune mechanistic models of auto-immune disease**
    - Immune, mechanistic, adoptive transfer, inflammation

14. **Animal models of autoimmune disease**
    - Autoimmune, disease, transplantation

15. **Mechanistic Models of Immuno-Oncology**
    - Cancer, Immunology

16. **Analysis of thymus development and function**
    - Thymus, tolerance, autoimmunity, development

17. **Induction, maintenance, and regulation of Type 2 Immunity**
    - Helminth, infection, allergy, tolerance, T cell

18. **Tuning the immune response in tuberculosis**
    - Tuberculosis, immune response, macrophage, zebrafish

19. **Non-invasive mucosal immunisation**
    - Vaccines, non-invasive, safety, efficacy, mucosal

20. **Regulation of autoimmunity in models of RA and SLE**
    - Rheumatoid arthritis, systemic lupus erythematosus

21. **Immune responses in transplantation therapies**
    - Transplantation therapy, Immunology, Regenerative medicine

22. **Testing of ES-62-based anti-inflammatory drugs**
    - Ageing; allergy; autoimmunity; drug development; parasitic worm

23. **Modelling and therapeutics for autoimmune disease**
    - Autoimmune disease, Gene Therapy, Adenovirus Vector, Autoimmune polyglandular syndrome type 1, Intrathymic injection

24. **Transplantation of regenerative cellular therapies**
    - Transplantation, stem cells, function, safety, immunogenicity

Project 1	**Development of hiv-1 vaccines**	
Key Words (max. 5 words)	HIV-1 vaccine, mice, immune responses	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
	x	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Despite remarkable progress achieved in decreasing HIV transmission and AIDS-related deaths in the last decade due to development of over 30 antiretroviral drugs, HIV continues to spread in a virtually uncontrolled manner. An effective HIV vaccine remains one of the priorities of HIV/AIDS research and will always be the best solution and likely key to any strategy for halting the AIDS epidemic. The biggest challenge in developing such a vaccine is the enormous HIV variability, which dwarfs that of any other virus except hepatitis C virus. However, all parts of HIV cannot easily change; to remain alive, HIV has to keep some smaller regions of its proteins more or less constant to maintain function. Our vaccine strategy takes advantage of this and focuses the body defenses on these conserved parts of HIV, its Achilles heel. Because conserved regions are common to most of the global HIV variants, the vaccine, if successful, could be used in Africa, Asia, Europe, America and Australia: it would be universal. The 1st generation conserved-region vaccine was very safe and induced strong immune responses in adult volunteers in the UK and Africa. The 2nd generation vaccine was constructed in 2014, using a new vaccine virus (ChAdOx1) and combining conserved regions with computer-designed proteins (mosaics), which significantly increase the vaccine	

	match to global HIV variants. (Note that even the highly conserved regions are still somewhat variable.) Vaccines should match circulating HIVs as much as possible to stop them efficiently. The 2nd generation vaccines have been constructed, shown to induce strong immune responses in animal models and are now bound for testing in human volunteers. This PPL will assist us to see how well the 2nd generation vaccine works in trying to clear HIV from already infected individuals and for larger scale testing in healthy (HIV-1 negative) volunteers in Africa. The PPL will also allow animal testing, which will enable evaluation of the most promising vaccine improvements in humans.
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)?	Effective preventive vaccine against HIV-1 can avert millions of new infections, empower women, protect children, circumvent the stigma facing men who have sex with men, and help many other beyond the reach of today's HIV-1 treatment and prevention options. Millions of already infected people around the word will benefit from HIV cure, either functional (drug-free control of HIV-1) or sterile (complete elimination of the virus from the body).
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use about 1,100 mice per year for 5 years. These may be inbred or genetically altered strains or outbred stocks.
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?	Typically we inject substances / vaccines intramuscularly, the same route as is intended for humans. The severity of our procedures is mild to moderate. All animals will be killed at the end of experiments. *1. Administration of substances* Appropriate induction and maintenance of pain relief and/or prevention of pain will be monitored throughout the procedure. *Short-term mild breathing and/or systemic illness* after recovery from injections or gavage is observed in less than 5% of animals. The health status of animals will be regularly monitored. Any animal showing signs of distress as a result of the injection will be killed. *Bleeding following intravenous injection* is a common adverse effect. This will be minimised by good operator technique and using the smallest needle

	practicable. Bleeding will be normally stopped by application of pressure. Any animal showing signs of distress as a result of the injection procedure will be killed. *Swelling resulting from walling off foreign substances* in the body may occur at the injection site, especially if substances enhancing immune stimulation have been used. In most cases, these will consist of a lump under the skin, which causes no pain and disappears spontaneously. Animals will be monitored regularly. In less than 5% animals, discontinuity or break in skin / a bodily surface (ulcer) may develop. Mice with swellings that have not healed within 48 hours will be killed. Mice that develop ulcers will be killed immediately. *2. Blood sampling* Adverse effect of blood sampling is decrease in the number of red blood cells in the blood and/or blood volume. Bleeding will be normally stopped by application of pressure. Blood sampling will not exceed 10% of the total blood volume in 24-hour period and will not exceed 15% of the blood volume in any 28-day period, with a minimum interval of 2 weeks between samples. Other rare adverse effects of puncturing veins include inflammation of veins, formation of blood clots or local collection of blood out the vessels (bruise). These will be practically ruled out by good operator practice, sterile techniques and appropriate staff training.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	*Vaccines have to be tested in living body:* The procedures described in this PPL cannot be done using cells, isolated organs or animals kept under general anesthesia. The vaccine-driven expansion of immune responses and optimization of delivery requires injection of candidate vaccines into a living body. The use of live animals is needed to study tissue distribution/homing of vaccine-generated T cells. *Human studies.* The past PPL supported testing of candidate vaccines in 10 phase I/IIa clinical trials in UK, Europe and Africa. There are a number of trials in the pipeline that will assess the safety and basic immunogenicity of the 2nd generation conserved mosaic vaccines in both uninfected and HIV-1-

	positive, early-treated individuals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Group sizes of animals will vary, but in all cases will be kept to minimum, which will provide statistically separable results. For new previously untested vaccines, the numbers will be initially low and the results will be used to design future statistically significant experiments. For induction of cell-mediated immunity by vaccination, unvaccinated control groups are typically not included as the background peptide responses in naïve mice are well established and minimal. Control groups will be needed ONLY for experiments determining protection against the surrogate virus challenge. In an effort to reduce numbers of mice used for vaccination studies, we have now implemented blood sampling as our standard method of immune monitoring. This allows to follow individual mouse responses throughout an immunization experiment and thus obtain data from multiple time points from one mouse. The main limitation is the blood volume/cell numbers recovered from a living animal. We shall use transgenic mice to gain insights into responses generated in humans. For these experiments, we shall only breed animals to meet our requirements. Validated potency tests for clinical trial vaccines will be carried out in groups of 5 mice. Based on the past variability of the assay, five, four or three animals fulfilling potency criteria give us 98%, 91% or 71% confidence (p=0.05), respectively, that the vaccines are fit for use.
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 are the lowest vertebrate group in the evolutionary tree for which suitable models of immune responses in humans and reagents are available. Some of the experiments will be carried out in genetically altered mice. In order to minimize animal suffering, particular attention will be focused on: 1 *The vaccine dose* For every new vaccine modality/vector, we shall start with a dose responses of the vaccine administration to established the minimum dose necessary to achieve desired levels of immune responses and do so with no or minimum

	side effects. These doses are then used for further experimentation. All vaccine dosings are done aseptically. 2 *Regular animal monitoring* to detect any change in behaviour, which may reflect pain, distress or discomfort. Any animal showing one or more of the following: (i) body mass loss greater than 15%; (ii) clinical signs and/or (iii) signs of malaise (e.g. hunched posture, staring coat) will be killed immediately by a Schedule 1 method. 3 *The use of the least severe procedures.* This is a simple, straightforward PPL where we plan to use the least severe reagents and methods. This is because we are developing vaccines and vaccination protocols, which are safe and ultimately acceptable for use in humans and indeed in human new-born babies.

Project 2	**Autophagy in the hematopoietic system**	
Key Words (max. 5 words)	Autophagy (self-eating), leukemia, autoimmunity, ageing immunity, vaccination	
Expected duration of the project (yrs)	5 yrs	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Autophagy is a cellular process by which cells dispose of and recycle unwanted material. This process has only been discovered about a decade ago, and most research has been performed on cells in culture. We now know that too little autophagy in blood cells can lead to cancer (leukaemia), failure to generate functional blood cells (differentiation), and immune deficiencies (inherited disease that makes patients susceptible to infections). Too much autophagy is also inappropriate as it leads to autoimmunity. Finally declining levels of autophagy with age result in poor immune and vaccination responses. It is unknown what exactly goes wrong when autophagy levels are altered. In this project we are firstly addressing the mechanism by which autophagy affects immunity and haematological malignancies. To date there are no adequate drugs with acceptable side effects which provide a defined mode of action to modulate autophagy in blood cells. Finding novel drug targets and drugs is the second objective of this project.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	This project will result in a better understanding of the cell biology of immunity (vaccines including in the elderly), of immune deficiencies, autoimmune diseases (lupus, IBD, arthritis) and haematological malignancies (leukemia). We hope to discover novel	

project)?	drugs to modulate autophagy in blood cells. We have already found that the naturally occurring drug spermidine induces autophagy and can rejuvenate immunity in the elderly. We will elucidate the mode of action of spermidine in blood cells, as well as determine the minimal dose to be given to have an effect, its distribution in the body and its side effects. These are all necessary steps for clinical trials in humans in the future.
What species and approximate numbers of animals do you expect to use over what period of time?	We will be using an absolute maximum of 11,600 of inbred mice over 5 years. Most of them will have a genetic modification.
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?	5000 mice are undergoing procedures on the mild protocol and will not experience any adverse effects. For the disease models we will be using (lupus, arthritis, skin inflammation, inflammatory bowel disease, and response to a pathogen) the adverse effects are expected to be of moderate severity. The main adverse effects induced by pathogens will be respiratory distress and weight loss linked to the induction of viral challenges, the main adverse effect of arthritis is swelling of the joints with pain and reduced mobility, adverse effect of inflammatory bowel disease is weight loss, diarrhoea and some pain, adverse effect of lupus is swollen abdomen, skin rash around the nose, accumulation of fluid under the skin and in the abdomen leading to a flabby swollen appearance, accompanied by pain and lessening mobility. Moderate severity is also in place for some of the proposed protocols to allow for the apparition of moderate adverse effects that cannot be predicted. Such effects may for example be due to the administration of substances that have not yet been tested/titrated in vivo and need to be used as a replacement/complement to the substances typically used or to genetic alterations leading to a particular susceptibility to the injected substances. Animals will be monitored throughout the course of the disease for the development of potential adverse effects and humane end points are in place based on the expected adverse effects Animals will be killed if they approach the severity limit. At the end of the experiment, all animals will be killed and tissues will be collected for further investigation.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are replacing animal experiments with in vitro cell culture models whenever possible. Examples of this is the use of samples from lupus patients, blood from ageing donors and from vaccinated donors. However, some experiments can only be done in vivo, for example there is no in vitro technique to replace the entire differentiation of blood cells from a stem cell. Secondly, the complexity of the interactions between the different cell types of the immune system cannot be adequately reproduced in a whole organism. Lastly dose, kinetics, side effects and tissue distribution of a novel drug need to be tested in an animal before its development as a drug for humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Here are some of the measures we are taking to reduce the number of animals: 1) Use of pilot experiments in small number of mice, especially for those procedures, for which there is no local or international expertise. 2) Obtain training elsewhere if expertise is not available 3) Taking into account our previous experience and those of our collaborators to determine group size 4) Optimization of extraction methods to provide sufficient numbers of particular cell types with minimum usage of mice 5) Inclusion of colonoscopy will allow individual mice to be followed throughout disease development for inflammatory bowel disease. This will reduce the number of mice required to look at the disease as it progresses 6) Maximising use of harvested cells and tissues: setting up a shared local aged mouse colony. 7) Archiving of frozen tissue samples to permit analyses of novel factors without additional in vivo experiments. 8) Embryo freezing of strains that are not currently in use. 9) Outsourcing to the expert: Generation of monoclonal and polyclonal antibodies will be outsourced when appropriate project authority exists
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	Mice and rats are the lowest vertebrate groups on which well-established models of immune and hematopoietic diseases of interest have been developed. Mice are preferable to rats because of the greater availability of reagents (e.g. monoclonal antibodies, tetramers) specific for this species and

measures you will take to minimise welfare costs (harms) to the animals.	availability of genetically modified mouse strains known side effects. We have determined doses and kinetics of immune modulatory chemicals and biologics in past experiments as we have many years of experience working on genetically modified animals. We will minimise welfare costs to the animals through rigorous model development approach and by continuously refining our protocols: through the use of pilot experiments, shortening the experiments to a minimum duration while still allowing a significant read-out and reducing dose and times of administration of substances. We have refined the genetically modified animal breeding, as most mouse models will only lack the cellular process of autophagy in a specific subpopulation of the blood system (neutrophils, T cells, B cells), which means that these mice will not develop disease unless their immune system is challenged. Furthermore the immune system challenge models have been carefully selected so that animals undergo the minimum pain. Number of injections will be kept to a minimum, and disease duration kept as short as possible as to still allow meaningful conclusions. Painkillers will be administered where they do not interfere with the disease process.

Project 3	**Immunity, tolerance & treatments in type 1 diabetes**	
Key Words (max. 5 words)	Immunology, type 1 diabetes, infection, autoimmunity	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Type 1 diabetes is a medical condition in which the body's immune system destroys the cells which produce insulin (beta cells). It requires lifelong management with insulin to control blood glucose. Type 1 diabetics have a reduced life expectancy and may suffer long term complications such as blindness, neuropathy, kidney disease and increased susceptibility to infection. The understanding of Type 1 diabetes has improved over the last decades, but the causes of disease and thus opportunities to prevent it remain elusive. The objectives of this project are to • **Gain further understanding of the causes and progression of type 1 diabetes.** We will achieve this through studies of the effects of genetic variation and of infectious agents on the immune system. • **Contribute to better treatment for type 1 diabetes.** We will achieve this through investigating insulin producing cell replacement therapy and combination treatments aiming at both stopping new insulin secreting cells from immune cell mediated destruction and restoring normal glucose control.	

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)?	Our aim is to understand the processes that lead to development of type 1 diabetes and to contribute to the cure and/or prevention of the disease. In the process we will produce genetically altered mice and protocols that will be useful and made available to the wider scientific community.
What species and approximate numbers of animals do you expect to use over what period of time?	30900 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 mice will not develop any adverse effects. In some cases study animals will develop diabetes. This is diagnosed by glucose in urine (as in humans) and in many cases the mice will not show any obvious adverse signs before being humanely killed. In some cases they will show adverse signs, such as subdued behaviour, hunched posture or weight loss. Such animals will be carefully monitored and humanely killed as soon as consistent with the experimental objective and in any case as soon as defined endpoints are reached (such as loss of 15% of body weight). Mice will be humanely killed by schedule 1 methods though a small number may be killed under general anaesthesia for the preservation of tissues for study. Genetically altered mice may be transferred to other projects with appropriate licence authorities to receive such animals.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Immune responses require the involvement of many cell types and organs, and usually the movement of cells through different organs over time. This can only be done in a living animal. However, we complement our animal studies with studies in the laboratory where mice are either not required or used only to supply organs/cells for further study.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We constantly adapt the group sizes used through power calculations based on previous results. We are also actively pursuing reduction of group sizes through development of live animal imaging of immune infiltration in islets. This allows us to monitor the same mouse throughout an immune response greatly reducing the numbers required to follow the progression of immune responses over time (days). When a mouse is killed, we regularly share tissues with our colleagues; this makes sure that as many

	tissues as possible are used and reduces the number of animals used in other projects.
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 NOD mouse develops spontaneous type 1 diabetes which shares many features with human disease. This makes it ideal for studying prevention and treatment of type 1 diabetes as well as autoimmunity in general. NOD mice can be genetically modified which greatly enhances our ability to learn about the mechanism of the disease and test strategies for its treatment. Diabetes development is monitored carefully. In those cases where we need to keep a diabetic mouse alive for experimental purposes we monitor its blood glucose and general condition, making sure it has dry bedding and enough water. If appropriate we also administer insulin injections.

Project 4	**Immune regulation, autoimmunity and infection**	
Key Words (max. 5 words)	Autoimmunity Infection Disease Prognosis	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	√	Basic research
	√	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	√	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	White blood cells of the immune system recognise and target foreign substances, including viruses and cancer cells. Autoimmune disease arises when these cells start to recognize and destroy normal healthy tissue. Our research aim is to understand what causes this switch to occur and how it might be reversed. Western medicine has developed by classifying disease into defined diagnostic categories (which gives each disease its different name), and understanding the biology that drives development of these specific diseases. This approach ignores the factors controlling disease course after diagnosis, despite the fact that this is what determines patient outcome. We have therefore focused Our research on understanding what controls long-term clinical outcome, or prognosis, in immune-mediated disease. This determines whether someone with a given disease has a mild course, or whether the disease is relapsing and progressive, causing disability and perhaps death. This could lead to personalised therapy, tailoring drug and treatment strategies to an individual patient's prognosis. We have evidence that some key white blood cells	

	that drive diseases such as inflammatory bowel disease, vasculitis and arthritis, exist in an exhausted state in some people, who then have a good long-term clinical outlook. Those whose cells are not exhausted have more aggressive disease. We will study this process to understand the pathways involved and finding drugs that manipulate exhaustion and can be used as new treatments for a range of diseases driven by the immune system.
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)?	We are focusing on how particular factors can influence the progression and outcome of a disease. By identifying and understanding these factors, it may be possible to use them to assist the prognosis of a disease at the time of diagnosis, which would have important implications for determining the treatment plan for individual patients. A better understanding of the exhausted state of the white blood cells in autoimmune patients with a good long-term outlook, may present the possibility of new therapeutic targets that may be effective across many different diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5 year course of the licence we expect to use an estimated 40,500 mice.
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 mice used on this licence will be genetically manipulated lines used for the purpose of breeding and *ex vivo* experimentation. Most genetically manipulated mouse lines will be healthy and show no deleterious effects. The mice are maintained in individually ventilated cages in a barrier environment to protect them and ensure any effect on the immune system does not compromise health of the animal. It is possible that a small number (approx. 5%) of genetically modified animals will develop an adverse effect which is of scientific interest, or will be prone to a spontaneous autoimmune disease. These animals will be regularly monitored and any mouse exhibiting two or more signs of distress, such as piloerection, subdued or unresponsive behaviour and hunched posture, or a 20% loss of previous recorded weight, will be humanely killed. The majority of the mice on this licence which undergo a regulated procedure will only ever reach mild actual severity, except in exceptional situations. This means than the mice will experience no more

	than transient discomfort and no lasting harm. In some circumstances, mice will be used for a disease model, either a model of autoimmune disease, inflammatory disease or in infection protocols. These mice may experience a moderate severity. The health of mice undergoing any procedure will be regularly monitored and any signs of distress noted. Any mouse exhibiting two or more signs of distress, as described above, will be humanely killed. The majority of animals will be killed by an appropriate humane 'Schedule 1' method at the end of the protocol. We have requested the use of non-schedule 1 methods, to allow us to take large blood samples or study short lived cell types which may be of interest, but it is expected these will only be very rarer used and in exceptional circumstances.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We plan to minimise the number of mice used by carrying out as much preliminary work as possible in vitro or ex vivo. These studies will allow us to optimize and focus our studies. Our combined approach, using human and animal data and state-of-the-art computational methodology, allows us to explore immunological mechanisms that are relevant to human disease, and to translate our results into applications of direct benefit to patients, for example biomarker development for better prediction of clinical outcome and new drug therapies. However, many aspects of the immune response cannot be studied in this way. Immune responses are complex and require a coordinated response from a large number of different types of white blood cell, and autoimmunity and inflammatory disease only develops in the context of these complex interactions.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We keep the number of mice bred to a minimum, centralising the administration of our strains to ensure coordination of different studies to prevent the duplication of experiments. Experiments are designed using statistical calculations to ensure results are informative and scientifically valid. We maximise the information we get from an individual animal by measuring the most variables we can from each mouse.
3. Refinement Explain the choice of species and why the animal model(s)	The parallels between the human and mouse immune systems are well understood, which makes the mouse a good species for these studies.

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.	Where possible we use studies which allow us to examine the immune system using tissue from dead mice, preventing direct procedures being carried out on live mice. We have been refining techniques, and have found alternatives for some protocols, such as the Collagen Induced Arthritis model, with improved induction requiring fewer injections and an alternative protocol to trial which can prevent ulceration. Some mouse models of autoimmune disease have been shown to vary with the genetic background of the mice. This can be manipulated using a genetic background with a less severe outcome for longer studies where possible, to reduce the overall severity for the mice involved.

Project 5	**Evaluation of Antibody Therapy in Immune Models**	
Key Words (max. 5 words)	Monoclonal Antibody, Immune therapy, Inflammation	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The main objective of this project licence is to understand how novel monoclonal antibodies, an antibody produced by a single clone of cells or a cell line and consisting of identical antibody molecules, which for example inhibit a cytokine (cytokines are small proteins secreted by certain cells which have an effect on other cells), modulate the immune system at a molecular and cellular level. This understanding will underpin the development of new treatments for inflammatory conditions. Despite the recent development of anti-cytokine antibodies, for the treatment of inflammatory conditions, there is still a need for the development of further treatments for these conditions as not all patients respond and current therapies still have notable toxicities. Many patients unfortunately still suffer from the clinical symptoms of these debilitating conditions which reduce both the quality of life and can reduce lifespan. With the data provided by this project licence we intend to develop new therapeutics for inflammatory conditions, bringing the opportunity of long term relief to a larger number of patients than can currently benefit from present therapies. We aim to generate new treatments, based on a class of drug called monoclonal antibodies. Our antibodies are designed to modulate the patient's immune response to the	

	inflammation. During the course of discovering new monoclonal antibodies, we will also be addressing the questions of which patients will respond and whether our treatments can help those who do not respond to the currently available therapies. This licence will also provide essential supporting data for all the antibody therapies we are developing, enabling us to determine the way our molecules are working.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The potential benefits of this project will be the development of new knowledge of the immune and inflammatory processes and the provision of data which will underpin the development of novel therapeutic antibodies leading to the progression of these new therapies into clinical development and ultimately onto the market bringing benefit to patients.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 11,000 adult mice over 5 years for this 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?	The majority of the adverse events expected under this licence (e.g. weight loss, pallor and hunched posture) will be associated with the immune models, models to evaluate the initiation, development and progression of the immune process, and the corresponding inflammation. The inflammation induced in these immune models may affect the whole animal, and can affect the general welfare of the animal. We have put in place measures to closely monitor the effects of this inflammation on animal health enabling us to monitor inflammatory response and its impact on the animal. Animal condition will be assessed daily and weight three times a week. Any changes in condition, weight and / or behaviour will be noted and animals deviating from normal condition and/or behaviour will be assessed. If necessary, following consultation with the animal care technician and /or the veterinary surgeon, mice maybe further closely monitored or an intervention such as the supply of dietary supplements may be given or animals may be killed. Adverse events can occur, following the administration of compounds depending on the intended mechanism of action of the molecules being tested. Where adverse events are noted, if necessary, following consultation with the veterinarian and appointed animal technician, mice

	maybe further closely monitored or an intervention such as the supply of dietary supplements may be given, additionally if it does not affect the outcome of the study and the severity limits are not exceeded then anti-inflammatory e.g. NSAID, short activing anti-histamines, or steroids can be administered to assist recovery, or animals may be killed. When mice are anaesthetised there will be close and continuous monitoring to ensure that there is no possibility of mice recovering consciousness until procedures have finished. At the end of the experiments, animals will be killed and tissues taken for further analysis. This analysis is an important and integral part of the project as this data will help us to decide which types of inflammatory response are likely to respond to our therapies and therefore which patients are most likely to benefit.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Inflammation is a complex process involving many cells types which interact with each other. This interaction is not just between immune cells but also between immune and non-immune cells. It is not currently possible to model these aspects and complex interactions without the use of animal models, as we cannot reproduce the overall complexity of the types of cells involved and how they interact with each other in an in vitro system. We will do this as we test our lead monoclonal antibodies in vitro first, only picking those that have the right characteristics to progress to in vivo models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will regularly consult the most current papers on the subject to make sure we have the most up to date scientific knowledge in the area of research we are working in. We will initially run studies with as few animals as possible to make sure that our experimental technique is correct and the models we have chosen are the most appropriate. In this way, for each project, only a few antibodies will need to be tested in vivo. We will also use statistical methods to ensure that we are using the fewest animals per experiment to obtain meaningful data. In addition we will be taking many samples which will tell us about the changes to the immune system at the end of each study to ensure that we gain the maximum amount of information from each animal.

	This will also minimise the number of experiments that need to be carried out. We will also intend to develop imaging techniques to enable us to monitor inflammation longitudinally which will enable us to use fewer animals and time points.
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 models have been successfully used for the development of inflammatory therapeutics for a wide range of inflammatory conditions. It is well established that antibodies can affect the inflammatory process and that these antibodies can then be used to treat patients with inflammatory conditions. It is also well established that as the immune cells in the mouse have a high level of similarity to humans and that mouse models of inflammation can predict the effects seen in inflammatory conditions (e.g. Rheumatoid Arthritis, etc.) in patients. Mice will be housed in state –of –the art conditions with care and welfare provided by an excellent and highly trained team of technicians. We will ensure that only agents that have passed stringent analysis for quality will be used in mouse studies. When conducting inflammatory studies we will select the protocol which uses the lowest concentration of antigen/cytokine etc. possible and has the least deleterious effect on the mouse during the course of the study whilst still meeting our experimental requirements. We will actively monitor the inflammation and the impact that it has on condition of the animal to ensure that no animal suffers beyond the severity limits allowed in the licence. We will continue to meet with local and international groups that work in the field on inflammation to refine experimental techniques and bring the best advice to bear on our projects so that we can always obtain the best information from the studies we run.

Project 6	**Coagulation Proteins in inflammation and immunity**	
Key Words (max. 5 words)	Inflammation, thrombin, immunity, atheroma	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our three objectives are to: a) Test novel anti-thrombotic compounds in models of vascular inflammation, in which we have previously showed that coagulation proteases play a critical role in pathophysiology. These compounds are being developed for clinical use. b) To further understand the cellular and molecular interactions important in the thrombin-dependent recruitment of inflammatory cells (including fibrocytes) to inflamed vascular wall. These experiments will yield new targets for therapy. c) To specifically address the hypothesis, in a mouse transplant model of chronic rejection, that fibrocytes mediate tissue fibrosis via replacement of microvascular endothelial cells.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This is predominantly translational work that has previously generated important insights into fundamental biological processes, to the benefit of the field in general – this is expected to continue in this new project. The specific benefits are potentially two fold. First, in this new project licence, we will be testing novel therapeutic compounds in our murine models that will have direct therapeutic application (and indeed, are undergoing pre-clinical development with a view to phase 1 clinical trials in the near future), so this new work might have direct clinical	

	impact. Second, we anticipate that there will be spin offs in the form of new projects (just as the atheroma project was a spin off from our transplantation work). In particular, under this project, and based on our identification of fibrocytes as important cells in intimal hyperplasia, we intend to assess whether coagulation proteins are involved in fibrosis, so there may be benefit in opening up an entirely new area of research.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse, approximately 600 per year.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The expected adverse outcomes relate mainly to complications of recovery surgery, required to induce inflammatory models, but animals suffering complications that we assess as severe will be killed by a schedule 1 method. The level of severity is expected to be moderate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	All animal work will follow on from in vitro testing. Our novel compounds have been tested to demonstrate pharmacological properties and we now wish to demonstrate efficacy in models of thrombosis and inflammation. Further dissection the cellular and molecular complexity of thrombin-dependent inflammation requires whole animal studies. Finally, tissue fibrosis has no appropriate in vitro models, so animals are required to study this process.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our experimental design is based on statistical advice, so that group sizes (n=6) will allow differentiation of a 20% difference between control and experimental arms at 90% power and 5% significance. We will collect all tissue, serum and cells from each animal and store in a local tissue bank to avoid having to repeat experiments more than is necessary.
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	Mice have been chosen because of the vast array of reagents and the number of genetically modified strains available. We will ensure that mice are appropriately monitored for specific complications, ensure that adequate analgesia is used, use anaesthetics appropriate for the species, and kill any mice assessed to be suffering.

minimise welfare costs (harms) to the animals.	

Project 7	**Mechanisms for Immunological Memory and Tolerance**	
Key Words (max. 5 words)	T cell memory, Regulatory T cell, Autoimmunity, Allergy	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The project aims to understand how a special type of blood cells, namely T cells, are produced in the thymus (the organ that is dedicated for the production of those cells), how T cells are maintained in other immune-related tissues, how these cells underlie mechanisms for vaccination and infection (i.e. immunological memory) and how abnormalities of these mechanisms can result in autoimmune and allergic diseases (i.e. breakdown of immunological tolerance). In addition, the project aims to provide new knowledge to the mechanism of autoimmune diseases, especially Multiple Sclerosis, for their prevention, diagnosis and treatment.	
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)?	Understanding how T cells are made to provide immunological memory and tolerance is important for the diagnosis and treatment of various diseases involving the immune system, including infectious disease, autoimmune disease, and allergy, and for the development of vaccines. Specifically, the project investigates the mechanism of how T cells react specifically to self-antigens (substances which induce antibody formation in another organism but to which the healthy immune system of the parent organism is tolerant), especially in the context of Multiple Sclerosis, and allergens.	

What species and approximate numbers of animals do you expect to use over what period of time?	Mice. We will expect to use up to 6000 animals over 5 years. 4000 of these animals will not be experimented on themselves, but will be involved in breeding or used as a source of tissues.
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 mice used during this study will not be subject to any adverse conditions, and be used as a source of tissue. Some mice will be used in experiments and may experience mild discomfort by immunisation or nutritional modification for a limited time. Some strains of mice will spontaneously develop autoimmunity and some mice develop autoimmunity as a result of immunisation. Our assessment is that these mice will experience moderate discomfort. At the end of experiments, these mice will be humanely killed and organs are taken for immunological and genomic analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	T cell tolerance and memory are controlled by different lymphocyte (white blood cells involved in immune response) populations and their interactions to each other, which can be analysed only through obtaining tissues and lymphocytes at the point when the immune system makes a decision on whether and how to respond to antigens. Currently, no in vitro assays or in silico (ie. using computer) method is available for this purpose, and also results that can be obtained by human patients are limited.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In order to minimise the use of animals, we will do the followings: (1) Pilot experiments. This allows us to carry out informative experiments only; (2) Power analysis (using results of pilot experiment and/or the literature) to obtain conclusive results with statistical significance using the minimum number of animals (statistical advice may be taken whenever required from collaborator statisticians); (3) Computational and pharmacological modelling, wherever possible, to determine and minimise the time points to be analysed. GM animals will be used, and this will allow more efficient and indispensable ways to achieve the scientific goals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	Mice are the most appropriate species, because they are the most established model for investigating immunological memory and tolerance, and have all relevant subtypes of lymphocytes for humans, and

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	have rich scientific resource such as antibodies. In addition, because GM animals are required for this project to investigate the gene functions, and mice have the broadest repertoire of GM available. The majority of the experiments in the project will be carried using mice as a source of tissue only. We propose to do mild procedures only, wherever possible. Thus experiments that induce mild discomfort (e.g. immunisation) only will precede and be prioritised to those that can induce moderate severity (e.g. autoimmunity). The part of the project that investigates autoimmune disease models (including the Multiple Sclerosis model) will focus on the investigation of the mechanism of the disease onset, and thereby minimise welfare costs to animals. Thus, animals will be humanely killed once the disease onset is confirmed (i.e. this will be either mild or moderate severity), and their organs and lymphocytes will be analysed. Skilled and experienced licensees will perform experiments. The animals will be housed in excellent conditions with appropriate bedding and nesting materials, and will be monitored and cared by professionally trained staff in the animal facility. Suffering will be minimised by maintaining mice under individually-ventilated cages and a specific pathogene-free (SPF) status to reduce the risks of infection; by regular and careful monitoring of animals after procedures; by monitoring mice for any signs of ill-health (e.g. ruffled-fur, weight-loss, behaviours including eating foods, changes in posture).

Project 8	**Macrophage biology in rodents and livestock**	
Key Words (max. 5 words)	Macrophage, Csf1, Csf1r	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The work outlined in this project is part of a long-term commitment to the study of macrophages, a type of white blood cell. Macrophages are important for the immune system and normal development. Specifically our aims are to: (1) Increase knowledge of general macrophage biology and their relationship to the rest of the immune system. (2) Examine the physiologic role of proteins (Csf1/Csf1r) which are important for macrophage development throughout the development & lifespan of an animal. (3) Determine/exploit the role of macrophages and/or Csf1/Csf1r during disease.	
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)?	Primarily this project is focused on expanding our understanding of the role of macrophages in immune function, growth and development, as well as health and disease. It is becoming increasingly clear that macrophages are influential in many physiological processes. Therefore, this is a significant area of scientific study which will contribute both to our understanding of basic physiology as well as potentially having therapeutic significance.	

	Immunotherapy is the treatment of disease by inducing, enhancing, or suppressing an immune response. The active agents of immunotherapy are collectively called immunomodulators which is an area of significant interest to us. By examining the effects of macrophage-targeted immunomodulators we will be able to: Increase our understanding of the cellular interactions and factors affecting growth and development. In the long term it may be possible to make use of these factors as part of genetic selection, improving production through genetics rather than therapeutics. In times of increasing food demand any factors that might safely improve growth are potentially of benefit. Our pilot studies involving potential new vaccines and gene therapy agents could be of benefit to both humans and animals in the long term.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse 850 Rat 750 Pig 100 Sheep 100 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 our experiments involve injection of substances which are not expected to have any adverse effects and would be classed as a mild severity. Surgical procedures would be classed as moderate severity but are all performed under general anaesthetic and analgesia is provided. All experimental animals will be humanely killed at specific time points in order for us to study the effect of these experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We use freshly isolated macrophages from humanely killed animals to investigate macrophage properties *in vitro* and we will continue to use this approach wherever possible and scientifically valid. However, *in vitro* assays cannot adequately model the complete role of macrophages during development or inflammation as macrophages are part of a massive number of cell types involved in inflammatory response and immunity. Therefore, an entire body model is required and further *in vivo* work is required.

2. Reduction Explain how you will assure the use of minimum numbers of animals	Our use of *in vitro* methods (see above) limits the numbers of animals required for the *in vivo* investigation stage. The proposed experimental designs and methods of analysis of the results will always be discussed with a statistician. We will use the smallest number of animals necessary to generate the scientific outputs. Animals will be made available for use on other scientific projects. Breeding will be optimised, wherever possible, to produce only the required animals of a particular genetic status. By freezing sperm and embryos to archive lines we will avoid wastage from the need to maintain colonies by continuous breeding.
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.	Much of our work is based on standard protocols but in order to optimise our studies we will research recent literature and seek veterinary and scientific advice where required. We will use pilot studies where necessary in order to maximise the information that can be gained from each animal and study whilst minimising adverse effects. The use of fluorescent reporter animals is a significant refinement as injections of substances is not required to visualise the cell. This also allows us to use fewer animals as we can specifically focus on the role of macrophages in these animals. We are familiar with many of the models described and have already refined some of the protocols to ensure that we induce a mild inflammatory response rather than clinical disease.

Project 9	**Development of the neonatal immune system and links with disease**	
Key Words (max. 5 words)	Immune system, neonatal, disease	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	It appears that babies may protect themselves from infection (a role of the immune system) in a different way to adults using different cells and different mediators. To try and understand this, we shall look at the development of cells that make up the immune system and how they work to see if this is different as animals' age. As cells of the immune system develop, some may not develop normally and this can result in certain leukaemias. The incidence of leukaemias has increased by just over 40% since the 1970s. T cell acute lymphoblastic leukaemia (TALL) is one such leukaemia and we will look at how this grows in mice and how we can reduce/prevent this growth.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	There are potential benefits from this research for both the scientific community and for disease treatment/prevention. Many babies (both those born early and at term) die from infection particularly from infections that have little effect in adults. Understanding how infants respond to these infections may help us better protect infants during early life Similarly, if we can understand which factors help T-ALL grow, this may open up an exciting new therapeutic avenues to try and halt the disease.	

What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice of different ages (from a few days old to adulthood) to study these questions. We will use around 6000 mice in total during the period of this licence (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?	Many animals used not be treated in any way, but will solely be used to provide different cells and tissues from mice of different ages and will experience no ill effects. Some mice will be given very mild doses of infectious agents leading to very minor effects on the animals who will be ki!led not long after the infection begins. Some mice will be given samples of human T-ALL and may experience some ill effects but these will be monitored and no mouse will experience any severe effects as they will be culled before reaching this point. All animals will be culled at the end of the project.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no way to monitor the different cells and mediators made in normal human children at different ages as these babies are not in hospital and so no blood can be taken. To follow this, we must take samples from mice at different ages. Similarly, leukaemia samples taken from people will not usually grow in the laboratory but only in an animal host. This project allows us to identify factors that may perturb or inhibit growth of leukaemia by following its growth in mice giving various treatments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	By careful analysis of every experiment we plan to undertake we will ensure we use the correct statistical design. This will mean we do not use more animals than is absolutely required to get a meaningful result. Where there are alternatives to these experiments and we can do experiments in the laboratory, we will do them.
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 are the best model to use as these can be easily modified in the laboratory and by far the majority of reagents are designed for use in this system meaning more useful data can be obtained from every mouse. Individual mice can also be followed over time to reduce the number of animals used in total. All experiments will be designed to produce the best results with the minimum severity to the animal. All animals will be extensively monitored and watched so that any ill-effect will be picked up immediately.

Project 10	**Antibodies for research and clinical purposes**	
Key Words (max. 5 words)	Monoclonal antibodies	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objective of this project is to produce antibodies to key targets across wide spectrum of species. Currently, there is evident lack of antibodies across a wide spectrum of organisms, especially in lower vertebrates. There is a growing interest in lower vertebrates among the scientific community as some species are being used as models for human diseases (e.g. zebrafish) and others have huge commercial values (e.g. salmon). Additionally, there is shortage of clinical antibodies which can be used for disease diagnosis and treatment.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The potential benefits of the project relate to providing antibodies for research purposes to proteins (targets) that otherwise have no available antibodies. Through the course of the licence we have developed 70 antibodies primarily to targets relating to human cancers and immunological conditions. This will aid the advancement of science in these areas as antibodies enable researchers to better understand the different functions and roles played by these proteins. The antibodies will be used to characterise the expression profile of proteins in normal and disease tissues thus enabling researchers to produce novel therapeutic treatments. Furthermore, the project is also expected to identify and validate disease markers in human, fish	

	and other vertebrates. The expected benefit will be the production of diagnostic and therapeutic products to improve clinical and veterinary management practice.
What species and approximate numbers of animals do you expect to use over what period of time?	During the 5 year duration of the project the cost to the animals of the proposed research is estimated to be 3 to 4 mice per month. The procedures to be used on the animals have been classed as mild. The reagents arising from the programme are invaluable for future research activities (e.g. discovery of anticancer therapeutics) therefore maximum output is gained through minimal discomfort to the 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?	Animals will be injected with desired immunogens using well established immunisation protocols designed to obtain a maximum response but to minimise animal discomfort. Blood samples — typically one per animals — will be taken Severity is mild discomfort. Animals are killed by schedule I at the end of the protocols.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Live animals have to be used since an active immune response is an essential prerequisite for the production of murine monoclonal antibodies. A significant amount of our work is carried out in silico and in vitro and hot in vivo as mentioned in the project plan. 20% of the work is carried out in vivo compared to 80% carried out in silico and in vitro.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Reagent companies and research centres use 2 mice (sometimes up to 5 mice) to generate one monoclonal antibody. However, due to our previous experience in this field and the use of in silico techniques, we are now able to reduce the number of animals to 4 antibodies per animal which represents at least 8 fold reduction in animal number used. In silico techniques using sophisticated computer algorithms are used to optimise and improve the peptide design of substances to be injected. An improved peptide design ensures increased success rates in production of antibodies which subsequently reduce the number of animals used. Further reduction in animal used is due to generating ONLY monoclonal antibodies which are stable cell lines producing unlimited stocks of antibodies, compared to polyclonal antibodies which are limited in amount and once antibody supply is exhausted further

	animals need to be used.
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 have well established protocols in place at the company for the generation of antibodies. Due to our previous experience in this field, only one blood sample is taken normally after the third immunisation to test for antibody titre compared to multiple blood sampling by other companies and research institutes. Complete Freund adjuvant (CF) and incomplete Freund adjuvant (IFA) were used throughout our previous work. FA has been shown to be very effective in terms of reliability and success particularly in relation to smaller compounds such as peptides and it is considered to be the standard adjuvant of choice in antibody production. All steps are carried out by experienced personnel at the designated establishment who are experienced and competent to handle the animals and perform the procedures. At all times the mice will have good handling to minimise discomfort. Any animal showing a significant deviation from normal health will be promptly and humanely killed or the NVS consulted.

Project 11	**Production of Antibodies**	
Key Words (max. 5 words)	Monoclonal, polyclonal anti-sera	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	√	Translational and applied research
		Regulatory use and routine production
	√	Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The purpose of this project is to enable polyclonal and monoclonal antibodies that are not readily available commercially to be produced effectively with the minimum number of animals for clients as a service.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The benefits to be derived from the proposed studies relate to the many applications of antibodies, from diagnostic assays to physiological studies and for specific drug targeting investigations. The use of antibodies is extensive and almost every scientific and medical area has felt their impact. Antibody-based diagnostics are now routinely used in medicine, veterinary science, agriculture, environmental monitoring and food safety evaluation. In addition, they may be employed for *in vivo* imaging of organs and tumours, and for targeting drugs to specific sites in order to obviate inappropriate side-effects. The benefits to be derived from the proposed studies relate to the many applications of antibodies, from diagnostic assays to physiological studies and for specific drug targeting investigations. Many diagnostic assays have been produced contributing to our understanding of brain peptides that control fertility.	
What species and	Maximum numbers over the five years but depends	

approximate numbers of animals do you expect to use over what period of time?	on demand: Rabbits, adult, 25 Mice, adult, 1000 Rats, adult, 250
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 purpose of this project is to provide polyclonal and monoclonal antibodies to clients that are not readily available commercially. During the course of the project animals will receive several injections, have blood samples taken and when the antibody level is at it's highest the animals will be humanely killed and a larger volume of blood removed for analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To date there are no viable alternatives for antibody production and animals remain essential.
2. Reduction Explain how you will assure the use of minimum numbers of animals	There is no prior hypothesis with antibody production, therefore, only the minimum number of animals will be used to get a sample for the purposes. In order to reduce the numbers of animals used, we have begun developing a predictive computer tool that allows better formulation of the substance to be injected into the animals – this will allow us reduce the numbers of animals/injections used as we will be more likely to raise high levels of antibodies.
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.	Rabbits, rats and mice are to be used for polyclonal production and mice only for monoclonal production. Adjuvant use is appropriately controlled to help minimise any potential adverse effects. Good handling of animals and antigen administration technique will minimise discomfort and harm to the animals.

Project 12	**Service monoclonal and polyclonal antibody production for Biological & Biomedical Research**	
Key Words (max. 5 words)	Polyclonal and monoclonal antibody production	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The purpose of this SERVICE project is the production of specific monoclonal and polyclonal antibodies to order, which can be used to address multifarious biological and biomedical questions posed by academic and industrial researchers.	
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)?	High quality mono- and polyclonal antibodies are invaluable tools to study biological systems (across all kingdoms) and are vital for example, in identifying and validating new biomedical targets for therapeutic benefit and also for use as therapeutic agents. This project will produce mono- and polyclonal antibodies to order supplying academic and industrial researchers spanning basic and translational research.	
What species and approximate numbers of animals do you expect to use over what period of time?	Over 5 years Rat 300 Rabbit 1110 Mice 500 Guinea Pig 200	
In the context of what you	The generation of antibodies requires the animals to	

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?	be inoculated with different biological agents (protein/peptides) that will stimulate an immune response. Appropriate anaesthetic regimes are utilised for the route of immunisation. Subcutaneous/muscular injection with or without electroporation is an expected mild severity limit. The selection of anaesthetic and application is suitable for the levels of discomfort expected. For intrasplenic injection the severity level is moderate because an incision is required to expose the spleen. The procedures may result in adverse effects but they are rare. Examples are granuloma (inflamed tissue) break down. This is managed by removal of the dead tissue and wound cleaning and the wound then heals normally. Blood sampling may result in transient discomfort but resolves quickly. Where an adverse effect does not resolve within a designated time period eg maximum 36 hr the animals will be humanely killed. In extremely rare circumstances anaphylaxis may occur, in this instance the animal is humanely killed immediately.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Antibodies are part of the immune system that recognises a foreign agent. An antibody is a complex molecule that can only be produced by the immune system of a living animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The object of the immunisation is to generate large volumes of high titre antibodies. Our immunisation regimes with multiple boosters for a longer period of achieve this. The longer time period also enables the spleen to become primed with the cell type necessary for *in vitro* antibody production. These techniques reduce the need for further animal use for that particular antigen. Using one spleen with an intrasplenic injection to generate monoclonal antibodies we can produce multiple hybridomas (fusion of antibody producing cell with a tumour cell) that are again used for *in vitro* antibody production. Getting the antibody into an *in vitro* production system removes the need for animal use. However, to get the initial antisera a living immune system is required as mentioned in replacement above. The introduction of RIMMS (repetitive, multiple site immunisation strategy) allows the production of novel

	antibodies in much shorter time frames and because of the methodology produce high affinity antibodies. These innovations reduces the number of animals necessary to raise antibodies because the technique produces better target specified antigens and also can use a wider range of immunogens to produce the antigens.
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 researchers who use antibodies sometimes need to use multiple antibodies in a study. If the different types of antigen are raised in the same species eg rabbit it is not possible to distinguish between them because the marker used to identify the antigen uses the species raised in as its target. Therefore, we have a range of species for polyclonal antibody production to allow multiple labelling studies to be carried out by researchers. The species chosen allow maximum titre and high affinity antibody generation. For monoclonal antibody production the spleen of the animal must be injected. We have refined our procedure removing the need for the invasive laparotomy (surgical incision in abdominal wall) to a small skin incision over the area where the spleen is found. The spleen is visible through the underlying muscle. This is a less invasive procedure and the animals are under anaesthesia for a much shorter period of time. The RIMMS refinement generates high affinity antibodies in a short period of time. Also this technique varies the route of antigen delivery maximising the presentation of the antigen to the animal, enhancing the response to the antigen.

Project 13	**Immune mechanistic models of auto-immune disease**	
Key Words (max. 5 words)	Immune, mechanistic, adoptive transfer, inflammation.	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project's objectives are to evaluate therapeutic entities in in-vivo mechanistic models, which are based around key aspects of immune mechanisms involved in human autoimmune diseases, including graft-versus-host disease and transplantation.	
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)?	Although many advances have been made in recent years in our knowledge and understanding of human autoimmune disease in identifying cell types and mediators involved in the mechanisms driving the disease process, a great unmet need for novel therapeutics that treat and cure autoimmune diseases still persist. This project will address some important immune mechanisms involved in human disease and the mode of action of novel therapeutics in modifying these mechanisms.	

What species and approximate numbers of animals do you expect to use over what period of time?	Rodents (mice, rats) will be used in this project. It is estimated that an average of 10390 animals could be used annually in this 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?	Animals may undergo procedures involving injections. The animals may experience short term discomfort caused by the injection. For all procedures anaesthesia will be used where appropriate and animals will be carefully monitored to ensure that discomfort to the animal is limited. All procedures have been ethically reviewed and all animals undergoing procedures will be well looked after by trained staff that work closely with a veterinary surgeon, At the end of each experiment all animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	At present, there are no alternatives, as in in vitro technology or computer modelling and simulations that can replace the requirement of the use of animals. Autoimmune and inflammatory processes are complex and involve many different cell types in the context of a microenvironment which cannot be reproduced in in vitro models. A better understanding of all of the components involved in these processes will help in establishing viable alternatives in the future.
2. Reduction Explain how you will assure the use of minimum numbers of animals	A significant proportion of drug discovery Is carried out using cells and cell lines (in vitro), with many thousands of potential drugs being screened to identify the most promising compounds. However, in order to study complex inflammatory responses, further testing is required in animals in which disease symptoms have been induced. To ensure the fewest number of animals are used, only the most effective drugs that have been pre-screened for activity in vitro will be examined in animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	Rodents are the lowest animals on the evolutionary scale in which disease processes similar to human disease can be generated. As our understanding of human disease increases animal models will be continually reviewed to ensure

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	that they are relevant to human disease. Selection of the most appropriate model to use to study a particular process in disease will be based on prior knowledge of that model. For all procedures anaesthesia will be used where appropriate and animals carefully monitored to ensure no animal experiences more than moderate discomfort. Humane endpoints are employed to limit suffering and burden to each animal. Substances given to the animals will be of a nature, route and frequency that of themselves will result in no more than transient discomfort and no lasting harm. All procedures have been ethically reviewed and all animals undergoing procedures will be well looked after by trained staff that work closely with a veterinary surgeon.

Project 14	Animal models of autoimmune disease	
Key Words (max. 5 words)	Autoimmune, disease, transplantation	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aims of this project are to identify effective new drugs for the treatment of chronic autoimmune diseases, such as Multiple Sclerosis, Rheumatoid Arthritis, Systemic Lupus Erythematosus, Inflammatory Bowel Disease and Psoriasis and also transplantation enabling the acceptance of grafts. Despite advances in the understanding of these diseases and their treatment, there is still a significant unmet clinical need. The purpose of this project is to mimic as far as possible the pathology of all these diseases and then to test the efficacy of our novel medicines in rodent models. To achieve this objective some basic research is required to further our understanding of these diseases and ensure that the animal models mimic aspects of the human disease process/mechanisms, whilst optimising and refining these models to ensure that distress and discomfort felt by the animals is kept to a minimum. Potential new drugs will be tested in these models.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or	This potential benefits of the project are (1) determining the *in vivo* efficacy (dose and exposure response relationships) of novel therapeutics to support their progression to clinical development, (2)	

animals could benefit from the project)?	the identification of novel drug targets, and (3) increased understanding of the immune response.
What species and approximate numbers of animals do you expect to use over what period of time?	The species used are mice, rat, guinea-pig and hamster with the following numbers 2200, 300, 50 and 100 used per year 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?	Reproducing aspects of autoimmune diseases such as Multiple Sclerosis, GvHD, SLE and Diabetes may lead to those animals displaying the signs and symptoms of the human disease. These are chronic debilitating diseases and in the majority of cases, this is anticipated to give rise to moderate discomfort. However in the case of Multiple Sclerosis and GvHD, it is anticipated that this may give rise to severe discomfort. This is required to ensure that the animal models effectively represent human disease pathology. Animals may undergo procedures involving injections. For all procedures anaesthesia will be used where appropriate and animals will be carefully monitored to ensure that discomfort to the animal is limited. All procedures have been ethically reviewed and all animals undergoing procedures will be well looked after by trained staff that work closely with a veterinary surgeon. At the end of each experiment all animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our current understanding of these autoimmune diseases and transplantation suggests that this is a highly complex process requiring multiple cell types and mediators that are necessary for a chronic inflammatory response. Consequently, it is difficult to model the disease process in its entirety using cell based systems and/or computer models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	A significant proportion of drug discovery is carried out using cells and cell lines (*in vitro*), with many thousands of potential drugs being screened to identify the most promising compounds. However, in order to study complex inflammatory responses, further testing is required in animals in which disease symptoms have been induced. To ensure the fewest

	number of animals are used, only the most effective drugs that have been pre-screened for activity *in vitro* will be examined in animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rodents are the lowest animals on the evolutionary scale in which disease processes similar to human disease can be generated. As our understanding of human disease increases animal models will be continually reviewed to ensure that they are relevant to human disease. Selection of the most appropriate model to use to study a particular process in disease will be based on prior knowledge of that model. For all procedures anaesthesia will be used where appropriate and animals carefully monitored to ensure no animal experiences more than moderate discomfort. Humane endpoints are employed to limit suffering and burden to each animal. Substances given to the animals will be of a nature, route and frequency that of themselves will result in no more than transient discomfort and no lasting harm. All procedures have been ethically reviewed and all animals undergoing procedures will be well looked after by trained staff that work closely with a veterinary surgeon.

Project 15	**Mechanistic Models of Immuno-Oncology**	
Key Words (max. 5 words)	Cancer, Immunology	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To identify drugs which activate the immune system to specifically eliminate tumour cells, thus aid the development of effective new therapies for the treatment of cancer. To achieve this objective some basic research is required to further our understanding of the complex interactions between tumour cells and the immune system. The effects of potential new drugs on the immune system will be tested in mice and subsequently in tumour bearing mice. It is critical these rodent experiments resemble human disease as closely as possible, whilst keeping any distress or discomfort felt by the animals to a minimum. Therefore an additional objective is to optimise these experimental systems. Current cancer therapies are often invasive, associated with substantial side effects and are too often ineffective. Cancer is one of the leading causes of morbidity and mortality in the world, causing 8.2 million deaths each year. Hence the need for new, effective medicines to treat cancer patients.	

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project is expected to identify novel immune processes involved in cancer, which will lead to new medicines for the induction and prolongation of remission in cancer patients.
What species and approximate numbers of animals do you expect to use over what period of time?	Only adult mice will be used in this project. It is anticipated that less than 1000 mice will be used each year for the 5 year duration of this 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?	Drugs targeting the immune system may cause transient discomfort for the animals. In addition some mice will be given tumour cells which will develop into a solid tumour. Tumours will not be allowed to grow larger than 1.5cm, or to spread throughout the body. Animals may also undergo procedure involving injections. For all procedures anaesthesia will be used where appropriate and animals carefully monitored to ensure no animal experiences discomfort exceeding moderate. All procedures have been ethically reviewed and all animals undergoing procedures will be well looked after by trained staff that work closely with a veterinary surgeon. At the end of each experiment all animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our current understanding of the immune system and how it interacts with tumour cells is insufficient to model disease processes in their entirety using cell based systems and/or computer models. These complex disease processes require all components of an immune system to be present to investigate the activity of novel drugs and to better predict the effects of that potential new treatment in man.
2. Reduction Explain how you will assure the use of minimum numbers of animals	A significant proportion of drug discovery is carried out using cells and cell lines (in vitro), with many thousands of potential drugs being screened to identify the most promising compounds. However, in order to study the immune system and the interaction

	between the immune system and tumours, further testing is required in animals. To ensure the fewest number of animals are used, only the most effective drugs that have been pre-screened for activity in vitro will be examined in animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the most appropriate mammalian species in which complex interactions between the immune system and tumour cells can be modelled and retain similarities to human. As our understanding of human disease increases animal models will be continually reviewed to ensure that they are relevant to human disease. Selection of the most appropriate model to use to study a particular process in disease will be based on prior knowledge of that model. For all procedures anaesthesia will be used where appropriate and animals carefully monitored to ensure no animal experiences discomfort exceeding moderate. Humane endpoints are employed to limit suffering and burden to each animal. Substances given to the animals will be of a nature, route and frequency that of themselves will result in no more than transient discomfort and no lasting harm. All procedures have been ethically reviewed and all animals undergoing procedures will be well looked after by trained staff that work closely with a veterinary surgeon.

Project 16	**Analysis of thymus development and function.**	
Key Words (max. 5 words)	Thymus, tolerance, autoimmunity, development	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The thymus serves as the primary site for the life-long formation of new T lymphocytes and is hence essential for the maintenance of an effective immune system. The complete lack of thymus tissue causes life-threatening immunodeficiencies typically hallmarked by infections, autoimmunity and/or increased susceptibility to cancer. Our research seeks to gain a better understanding of the development and function of the thymus and will form the rational basis for the design of novel diagnostic and therapeutic tools. The lack of knowledge concerning the molecular control of thymic epithelial cell (TEC) development and function prevents the development of specific therapies that correct the defect which cause diseases of TEC. A comprehensive understanding of TEC function will therefore help in the rational design of novel strategies to improve or correct the adaptive immune system in aged individuals and in recipients of haematopoietic stem cells, respectively.	

	Moreover, this knowledge may also foster the identification of novel biomarkers to be used as diagnostic tools for thymus function in humans. The project seeks to develop novel mouse models of human diseases and developmental processes. The work consists of three sequential stages: (i) generation of mice with TEC-targeted gain/loss-of function mutations; (ii) in-depth analysis of the animal's capacity to generate a regular thymus and a competent T cell compartment; and (iii) identification of the cellular and molecular mechanisms and consequences that lead to and are responsible for alterations in the physiology of regular thymus development and function using these specific experimental manipulations.
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)?	Insight gained from the outlined research program has several benefits as the knowledge gained will inform the creation of better diagnostic tools and novel therapeutic opportunities to correct thymus stromal defects in humans. Either of these benefits depends on the ability to establish in mice models of human pathologies for which specific mutations have been identified but the underlying molecular mechanisms not yet specified.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse, 32,000 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?	Irradiation will be used to destroy the immune system, which will then be rescued with donor cells. Surgery will be used to remove thymus tissue in wild type mice or to graft thymus tissues in mice with a inborn lack of a thymus. Animals will be immunised to study immune responses. Animals will be bred with gene mutations that will help us understand the development and function of the thymus.

	Blood and tissues samples will be taken to probe the cells of the immune system and substances will be given to investigate the function of T cells. All of the above could, but are not expected to, lead to infections, may transiently be related with pain, and will be associated for a short period with reduced well being as part of the recovery following surgery. Good surgical technique, pain relief, good husbandry, early intervention and treatment will be provided. All animals will be killed at the end of the experiment or as soon as an animal has been judged to have reached a humane end point, so that suffering is reduced. The expected level of severity for the protocols will be mild to moderate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Thymus function relies on the 3D organisation of its epithelial compartment, a structural organisation unique to the thymus and not found elsewhere in the body. All attempts until now to remove thymic epithelial cells and grow them as cell lines in culture have resulted not only in a loss of the 3D-organsation but also in a complete lack of function with regards to thymopoietic activity. *Ex vivo* foetal thymus re-aggregation cultures are possible but do not represent a suitable alternative for the following reasons: 1. T and thymic epithelial cell development cannot be followed completely; and 2. Comprehensive functional assessments of mature T cells are not possible. Therefore, these processes have to be investigated using animals. The procedures are limited to those that cannot be replaced by alternative methods, and the numbers of animals to be used are limited to the minimum number compatible with good scientific practice.
2. Reduction Explain how you will assure the use of minimum numbers	We will use the smallest possible group sizes still informative for thorough statistical analysis and logical progression to the next step only when necessary will ensure that a minimum of animals are used. Advice will be sought from biostatisticians.

of animals	Breeding will be set up so that the minimal number of animals is used to obtain the correct genetic crosses. Mice produced as part of the breeding that however do not have the correct genetic characteristics will be used as controls.
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 most widely used system to study the immune system and organogenesis. Mouse and human thymus organogenesis share conserved regulatory pathways. Antibodies for the identification and purification of different classes of cells; *in vitro* and *in vivo* functional assays; and inbred, knockout and transgenic mice are available. The mouse genome is phylogenetically closer to the human, compared to any other model organisms. The availability of complete genomic sequence, and extensive chromosomal synteny, makes the identification of homologous human genes considerably easier. Mice are the most appropriate small animal model for the study of the thymus as the biological, molecular and genomic information available for these animals is unparalleled when compared to other small animal models in biomedical research. In addition both structure and function of the mouse thymus is an excellent model for its human counterpart. Using tissue selective gene knockout targeted to thymic epithelial cells reduces the possible burden of broad organ malformations to gene targeted mice. The health and behavioural status of mice devoid of a normal thymus are not compromised when kept under specific pathogen free (SPF) conditions. Transgenic animals exhibiting any unexpected harmful phenotype will be humanely killed, Procedures will be minimised in frequency and adapted if more refined techniques should become available. All procedures that are likely to cause stress and pain will be done under appropriate anaesthesia and analgesia. The most refined surgical procedures and routes of drug administration will be employed.

Project 17	**Induction, maintenance, and regulation of Type 2 Immunity**	
Key Words (max. 5 words)	Helminth, infection, allergy, tolerance, T cell	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	Yes	Basic research
	Yes	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	Yes	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Parasitic worms (helminths) infect over 2 billion people worldwide resulting in a huge health and economic burden. The parasites this proposal focuses on are filarial worms, which infect 120 million people, and can cause diseases such as elephantiasis and river blindness. They survive within humans for decades, using a variety of strategies to manipulate and turn off immune responses that are responsible for killing infections. The immune system uses different types of immune response to deal with different types of infections. Type 2 immune responses are specialised for combatting infections with large parasitic worms. Unfortunately, if they go wrong, Type 2 immune responses can also cause diseases such as allergies. Understanding how these parasites turn off Type 2 immune responses is important for two reasons: (1) It will allow us to design vaccines that are resistant to parasite manipulation and turn on immune responses	

	to kill the parasites. (2) Some human diseases, such as allergies and autoimmunity, are caused by unwanted or incorrect immune responses. To cure these diseases we need to find new ways of switching off the immune responses causing them, and in these situations we can harness the strategies parasites use to subdue immunity. Thus, the objectives of our research are to understand how Type 2 immune responses are used to kill parasitic helminths, and how parasitic helminths are able to subvert and switch off Type 2 immunity.
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)?	Helminths infect over a third of the human population and the vast majority of wild and domestic animals. Although rarely fatal, they affect mental and physical development in humans and can cause severe debilitating disease. The also represent an enormous economic burden to the livestock industry. Our findings will help develop strategies, such as vaccination, for controlling these diseases. In particular, we study parasites that in humans cause elephantiasis and river blindness. Allergies affect 150 million people within the EU, and asthma affects 300 million people worldwide. This project will develop our understanding of how we can turn-off the immune responses that cause allergies. This will help develop new strategies for the treatment of allergies and asthma. The work we do is fundamental research designed to unravel the details of the interactions between cells of the immune system and the rest of the body. Therefore, we don't expect to generate or test therapies directly. However, the work we do will provide important knowledge for those involved in direct translational programmes.
What species and approximate numbers of animals do you expect to use over what period of time?	The majority of animals we will use will be mice. We expectto use approx. 12000 over5years. Approx. 3000 of these will be used for breeding strains that have specific gene modifications needed to test particular hypothesis. We will also use approx. 1600 gerbils, 100 rats, and 4000 mice to maintain life cycles of the

	helminth parasites including their intermediate hosts. 2000 mice will be used to investigate immune responses during infection, 1200 to study vaccination, and 400 to study allergic asthma.
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 animals will experience a maximum of moderate levels of severity. However, as helm inth infections are generally well tolerated and do not cause significant pathology, a large proportion of animals will experience no or mild adverse effects. Animals used for maintaining the intermediate hosts (vectors) of helminths will experience moderate severity related to exposure to biting mites. Experimental manipulations used to investigate how the immune system functions during infection and vaccination can result in moderate adverse effects due to increased immune inflammation. Models of allergic asthma can require repeated anaesthesia, and exposre to bronchorestricters to assess lung function, and result in lung inflammation leading to moderate levels of severity.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our studies rely on looking at the immune response to infection or other conditions in the context of the whole body. The immune response is a highly complex process involving multiple different cell types and molecules that work together. The function of these cells or molecules depends on where they are in the body, and often they move around the body. We cannot replicate these processes outside the body, No alternatives for exist for parasite migration through the body, and it is not possible to mimic helminth infection in vitro. Whenever possible, we use cell culture systems to address specific questions.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our experiments are carefully designed such that the results will be clearly interpretable using the smallest number of mice. We consult statisticians whenever necessary to ensure this is the case. Additionally, through careful assessment of animal strains used, we have over the past 5 years dramatically reduced the numbers of animals needed for maintenance of

	parasite life cycles.
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 a variety of helminth models that infect different tissues of the body. When the natural host is a mouse, the mouse is used to keep parasites alive (none can be maintained in culture). In some cases, rats or gerbils are more susceptible to infection and then these are used to maintain the parasite, so that we can use the fewest animals possible. Animals are closely monitored for any ill effects, typically by visual assessment and weighing. Weight loss can predict ill effects before they are seen visually. When performing experimental manipulations we will use the least invasive and distressing procedures available, and use the least number of manipulations, to reduce pain, suffering, distress, and lasting harm to animals.

Project 18	**Tuning the immune response in tuberculosis**	
Key Words (max. 5 words)	Tuberculosis, immune response, macrophage, zebrafish	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The human immune system can protect against tuberculosis (TB) but is also responsible for causing the tissue damage that may result from TB disease. I aim to use the zebrafish model of TB to increase our understanding of the parts of the immune system that influence the balance of beneficial and harmful responses to TB infection in order to identify new targets for more effective treatments and vaccines.	
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)?	I hope to gain new insights that help to develop novel interventions that will significantly shorten the length of anti-TB treatment, which will be important to reduce spread of TB and minimise development of drug resistance. I anticipate that this work may also lead to design of new vaccines that prevent TB disease altogether.	

What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 4000 zebrafish over 4 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?	There are no expected adverse effects. However, rare adverse effects may include abnormal swimming, infection and abnormal development or phenotypes. The likely level of severity is mild in all cases. Animals will be continuously used or killed according to a Schedule I method at the end of this project.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animal use is necessary for the proposed work to study dynamic interactions between the immune system and TB bacteria in a living host and to genetically modify components of the experimental system (knocking out or over-expressing molecules), which is not possible using human subjects or laboratory cell culture techniques.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All experiments will be performed on larvae before the onset of independent feeding; these larvae are not considered protected by the Animals (Scientific Procedures) Act. However, a licence is required for maintenance and breeding of adult fish to produce embryos for the proposed experiments. Animals will be raised according to a protocol that has a high survival rate, reducing the number of animals generated overall. Sperm harvesting and freezing (cryopreservation) will help minimise the number of animals produced as unused lines can be archived and regenerated when required, with no need to continuously maintain lines.
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	The zebrafish Mycobacterium marinum infection model provides a natural host-pathogen pairing which leads to inflammation that is an excellent model for human TB. Importantly, previous observations using this model have translated directly to human TB and provided important insights that may lead to improvements in treatment of tuberculous meningitis. The zebrafish is an accurate model of the mammalian

take to minimise welfare costs (harms) to the animals.	immune system, in which levels of factors that influence the immune response can be genetically manipulated to assess their function. In addition, immature (larval) zebrafish are transparent, allowing bacterial growth and cellular responses to infection to be visualised. All experiments will be performed on larvae before the onset of independent feeding; these larvae are not considered protected by the Animals (Scientific Procedures) Act. Adult zebrafish will be used to generate the larvae required for experimentation. These fish will live a normal, healthy life as any aquarium fish. Animals will be raised according to a protocol that has a high survival rate, reducing the number of animals generated, as well as unnecessary suffering and fatality. Fish will be closely monitored by highly skilled staff to ensure that animals are bred at safe intervals and when it is beneficial to their health (preventing egg-bound females). In line with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines, once experiments have been performed using a required fish line, sperm will be frozen and stocks will not be maintained further. Sperm harvesting and storage minimises numbers of live fish generated/maintained but does require use of anaesthetic and handling, which may be stressors. However, current techniques do not require death of the fish, and are performed by highly skilled staff trained in these procedures, minimising stress to the animals.

Project 19	Non-invasive mucosal immunisation	
Key Words (max. 5 words)	Vaccines, non-invasive, safety, efficacy, mucosal	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	√	Basic research
	√	Translational and applied research
		Regulatory use and routine production
	√	Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objective of the project is to develop mucosal vaccines that are safe and efficacious. Mucosal vaccines enable a two-pronged immune protection – systemic and at mucosal surfaces – compared with parenteral vaccines. The pharmaceutical industry still has the desire of producing the next generation of vaccines as mucosal ones and a better understanding of these types of vaccines is required.	
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)?	Mucosal vaccines will be formulated to protect against infectious disease and to prevent non-infectious conditions. This will be achieved primarily by studying: 1. The effects of novel delivery systems, in terms of improving antibody and cellular responses to particular antigens. 2. The mechanism of action of the novel delivery systems. The aim will be to reduce suffering in vaccine *in vivo*	

	studies and to produce formulations of commercial potential to alleviate suffering in animals and humans.
What species and approximate numbers of animals do you expect to use over what period of time?	We would expect to use 500 mice and 500 rats.
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?	Any adverse effects are most likely to occur due to administration via a specific mucosal route. In all cases these will be mild in severity and can be reduced by good handling to minimise discomfort. Anaphylaxis caused by antigen is possible but rare. No adverse effects expected from pre-treatment (eg ranitidine). Any animals deviating from normal health will be terminated humanely. Boosters will be no more in number than required to achieve and maintain the required titre. Animals that fail to respond within four boosters will be withdrawn from the protocol. Animals will be sacrificed humanely at the end of the study to obtain blood and tissue samples. Expected adverse effects for specific routes are detailed below: Subcutaneous injections: Minor swelling and erythema at the site of injection, with major ulceration expected in 1:1000. Intramuscular injections: Mild swelling and erythema at injection site. Some discomfort at the injection site and lameness observed in less than 1 in 1000 administrations. Buccal: Mild irritation may occur in mouth lining. Oral dosing/gavage: Minor discomfort at time of dosing, rarely the substance may enter the airway or the oesophagus is damaged. Substance in airway or oesophageal damage in less than 1 in 1000 administrations. Intranasal: Short lived nasal irritation. Increased respiratory rate. Increase in respiratory rate is observed in less than 1 in 1000 animals. Topical: Mild erythema. Possibly mild swelling and discomfort. Mild erythema might be seen in all cases.

	Swelling observed in less than 1 in a 1000. Vaginal: Short lived mucosal irritation. Inhalation: Animals will be treated by inhalation using a nebuliser. Mice will be treated without using an anaesthetic using a Volumetric Spacer or a suitable commercial holder. Rats will be treated by direct nebulisation with/without an anaesthetic or by using a Volumetric Spacer or a suitable commercial holder.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The immune system and interaction of different cells, signalling and antibody responses cannot be predicted by *in vitro* assays. The Regulatory Authorities are researching parameters that can be used in *in vitro* assays, but until these are established, animal experimentation is necessary. It may be possible to correlate data from *in vitro* and *in vivo* experimentation so that in the future, replacement can be achieved. *Ex vivo* studies are planned to replace some experimentation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Discussion with a statistician has enabled us to choose the appropriate number of animals that should be used per treatment and control group. This type of work is a pilot study and does not have a prior hypothesis to determine the sample size. However for an observational study such as this, comparing antibody and cytokine levels, 5 animals per group are considered adequate to estimate means and variability and to allow for loss of data (ie if an animals needs to be sacrificed before the end of the study).
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	Pilot studies are planned wherever necessary. Good handling to minimise discomfort and at least daily observation after dosing. Euthanasia if signs of mis-dosing (hunched posture, inappentance, reluctance to move) or lethargy/diarrhoea persist. Animals will be maintained on soft bedding. Good techniques to minimise discomfort after bleeding will be adopted, however, any animal showing lethargy and extreme pallor will be euthanised. Veterinary advice will be

(harms) to the animals.	sought where and when necessary.

Project 20	**Regulation of autoimmunity in models of RA and SLE**	
Key Words (max. 5 words)	Rheumatoid arthritis, systemic lupus erythematosus	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The work carried out in this laboratory will find out whether specialised cells called regulatory cells can stop autoimmune rheumatic diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), unravel the mechanisms by which they suppress disease, study how new treatments enhance the function and numbers of regulatory cells and identify novel therapeutic targets. Our laboratory has a long-standing interest in using regulatory immune cell subsets (such as regulatory T and B cells) for the down-modulation of autoimmune diseases. Our overall aim is to develop therapeutic agents, which can modulate anti-inflammatory cells and so lead to reduced symptoms and long-term remission from autoimmune disease. We will also study the role of cells that die during inflammation and how these cells can control regulatory cells. The process of switching off	

	inflammation by dying cells could be vital to curing diseases such as RA and SLE.
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)?	Ultimately we seek to improve the management of patients suffering from SLE and rheumatoid arthritis through greater understanding of the causes of these complex diseases. Interventions in human patients must clearly better targeted to avoid the side effects of the broad spectrum immunosuppressive therapies that include the in creased risk of infection and bone marrow suppression. Lupus remains a potentially serious disease; of the 396 patients we have followed over the past 25 years, 14% have died, with an average age of 57 years. Rheumatoid arthritis affects 0.8% of the UK population, is a highly debilitating disease, and patients have a reduced life expectancy. This research will improve our understanding how autoimmunity develops and therefore how it can be treated.
What species and approximate numbers of animals do you expect to use over what period of time?	The species to be used is mouse, and we expect to Use about 2000 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 mice will develop arthritis or signs of autoimmunity which means that they will experience some degree of pain, suffering, distress or lasting harm, but steps will be taken to minimise this. Mice will be killed humanely at the end of the experiments or if they reach clearly defined severity limits.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The study of cells isolated from patients and studied in test tubes leads to inconclusive and often irreproducible results. For these reasons we propose to use animal models for autoimmune disease, which closely mimic human diseases like RA and SLE. These models are fundamental for dissecting and understanding many of the basic immunological mechanisms driving diseases. Much of our previously published work in this area has focused on studying

	the blood of patients with SLE and RA, however to truly understand the mechanism of immune suppression by regulatory cell populations, we now need to combine our in vitro studies using patient samples with mouse models of disease. Whenever possible we will take advantage of available cell lines or patient samples, thus reducing the numbers of mice used.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Careful consideration is given to the minimal number of mice needed to give statistically significant results. Group sizes of 5 to 10 are necessary to show statistical significance. The size of the group has been decided after consultation with the statistician and we will continue consultation for optimisation of the experiments throughout the life of the licence. All our in vivo studies are followed by extended ex vivo analysis (we study lymph nodes, spleen, liver etc) thus again maximising the results and minimising the number of times that we have to repeat the experiments in order to reach statistical significance. Wherever possible, preferential use of the AIA model, instead of the CIA model, will allow us to obtain more reproducible results and to minimise the number of animals required to reach statistical significance.
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.	Different mouse models each offer distinct biological advantages. For example, the collagen induced arthritis (CIA) disease model is considered the gold standard model to test new therapies. This model, unlike other models of arthritis, mimics the acute and the chronic phase of the human disease. Antigen Induced arthritis (AIA) is, in contrast, used to dissect immunological pathways driving the phase. of disease. Mice affected with AIA develop a localized inflammation in the joints, which resolves spontaneously. It is important to understand the mechanisms driving the full resolution of disease in this model as they could be then translated into the human setting. In addition, 100% of mice immunised for induction of AIA, develop highly reproducible arthritis. This is in contrast with less than 80% of mice developing arthritis in the CIA model. Mice will be monitored carefully for ulcers and treated

	with topical creams; for arthritis to ensure the severity limits are not exceeded; and for infection and treated appropriately with antibiotics. Mice with arthritis will be provided with floor feeding and soft bedding. All animals are checked daily by animal technicians who are aware of the above adverse effects and will contact the user immediately if these are suspected. If any animal exhibits signs of distress (such as hunched appearance) it will be killed using schedule 1 methods. Bleeding after blood sampling will be controlled by local pressure and application of tissue adhesive/sealant. Blood sampling will not exceed maximum 10% Total Blood Volume (TBV) in 24 hours or 15i% TBV in any 28 day period.

Project 21	**Immune responses in transplantation therapies**	
Key Words (max. 5 words)	Transplantation therapy, Immunology, Regenerative medicine	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Transplantation is currently the treatment of choice for many forms of organ failure. The procedure, when successful, prolongs and improves the quality of life. However, the immune system of the recipient of the transplant recognises the foreign organ, and its uncontrolled response will lead to transplant rejection. To control rejection patients have to take medicines that suppress the immune system. Thus, these medicines make the patient more susceptible to infection and cancer. The overall aim of this project is to gain a greater understanding of how the immune system reacts to foreign tissues, especially transplanted organs. This further understanding can lead to new treatments to make the transplant last longer in the absence of any detrimental medication.	
What are the potential benefits likely to derive from this project (how science	The discoveries of the previous licence created a platform for the first clinical trial using adoptive transfer of recipient cells with regulatory functions, manipulated	

could be advanced or humans or animals could benefit from the project)?	in special laboratory, in solid organ transplantation. The work on this licence lead to new clinical trials aiming to improve the quality of live of transplant patients. We have learnt along the way and the information arise from our studies in mice together with clinical trial results will be used to refine protocols for the upcoming clinical trials using these cells to increase transplant survival in the absence of detrimental medication which in turns will also benefit the NHS.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, up to 12,000 in the 5 years timeframe.
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 our procedures do not exceed a level of severity considered moderate. Surgical procedure are performed under general anaesthesia and using painkillers to avoid post-operative suffering. Mice recovered very fast they do not show signs of discomfort. At the end of the experiment mice are culled to analyse all the immunological parameters. We have also a protocol to study graft-versus-host diseases, where the immune system attacks the recipient. These mice are followed closely and if mice are suffering (loss of weight or hunched or lack of movement) they are culled immediately.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We will use in vitro assays as much as possible to replace in vivo experiments. Initially, in vitro and ex vivo assays may provide insight into the cellular mechanisms involved in allograft rejection. However, they cannot adequately model the complete array of immunological factors and cells that are important in vivo. Therefore, further in vivo work is required.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The sizes of experimental groups and the number of repeated experiments will be kept to a minimum while ensuring that reproducible results are obtained with clear biological significance. We were able to optimised protocols and reduce 35% the use of mice in our previous licence and we expect to continue reducing

	the number of 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.	Mice have been chosen as the experimental animal of choice as they have an immune system of comparable complexity to the human. For these reasons, mice are the most frequently used animals in studies of the human immune system. This project aims to breed transgenic mice for use in biomedical research. These animals are needed to investigate the role of different immune cells and the influence of genetic factors in a transplantation setting. The humanised mouse protocols developed to study transplantation rejection have been selected as they allow the engraftment of human tissue such as human skin or vessels, as previously demonstrated by others and us. Combined with the successful engraftment of human cells and reconstitution of a functional human immune compartment, the humanised mouse models provide an important bridge to study in vivo efficacy of transplant tolerance induction strategies in humans. The humanised mouse model is a refined model per se that allow us to investigate important question, mimicking some of the human complexity without the need of non-human primates.

Project 22	**Testing of ES-62-based anti-inflammatory drugs**	
Key Words (max. 5 words)	Ageing; allergy; autoimmunity; drug development; parasitic worm	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project is concerned with a molecule that I discovered in the secretions of a parasitic worm, which due to its unusual structure, has anti-inflammatory properties. The project's primary aim is to determine whether novel synthetic drug-like Small Molecule Analogues (SMAs) of the worm product can protect against the development of allergic conditions such as asthma, autoimmune diseases like arthritis, and illnesses associated with unhealthy ageing, for example, cardiovascular disease. The plan is to employ mouse models of these important diseases to determine whether the SMAs can represent a starting point for novel drug development.	
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)?	It is currently estimated that >10% of the Western population suffer from allergic, autoimmune and/or unhealthy ageing conditions and this number is increasing at an alarming rate. Explanations for this increase include lack of exposure to pathogens in the West due to increased hygiene in combination with	

	vaccination resulting in allergy/autoimmunity causing abnormal development of the immune system during childhood (the “Hygiene Hypothesis”) and a Western life-style incorporating high-fat diet and/or lack of exercise causing unhealthy ageing. Consistent with the former suggestion, there is epidemiological evidence that parasitic worm infection may protect humans against the development of allergy, autoimmunity but in addition, cardiovascular diseases. Current treatments for these conditions involve the use of drugs that suffer from problems such as unwanted side effects or limited efficacy and indeed there is absolute failure to improve the condition of some patients. The idea of using molecules based on anti-inflammatory worm products represents a novel form of therapy. The aim of the research is thus to test the molecules in laboratory models of allergic, autoimmune and ageing conditions and if successful, to establish their mode of action. Overall, the program is designed to provide urgently required novel treatments for diseases that affect large areas of both the Industrialised and Developing World and whose incidence has been increasing over several decades. These diseases do not simply cause morbidity: around one quarter of a million people die annually from asthma worldwide, it is becoming increasingly apparent that rheumatoid arthritis is associated with reduced life expectancy, and cardiovascular diseases constitute the major cause of death of humans.
What species and approximate numbers of animals do you expect to use over what period of time?	A maximum of 6,550 mice will be employed in the work over the five-year period of the project. However, the actual number is likely to be much less than this as for example the number of compounds to be tested may not be successfully generated (due to failure to pass pre-animal preliminary screening tests in the laboratory) and compounds proving unsuccessful in initial mouse experiments will not be tested in repeat experiments. The project will also require around 600 jirds (gerbils) for maintenance of

	the parasitic worm.
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 expected adverse effects reflect the model being employed. Some of these, for example when investigating cardiovascular disease, are classified as “mild” in that there is no obvious indication of the animals suffering stress. However, others mimic the human situation, for example animals with arthritis may suffer some pain associated with inflammation. Such animals are monitored closely and using established guidelines that are strictly adhered to. Analgesics can be administered to alleviate such pain but if a mouse is considered to have a level of inflammation as determined by joint swelling that is above the accepted level, it is humanely killed. All animals in all experiments are likewise humanely killed at the end of the experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In order for the program of work to succeed, animals (jirds) have to be used to provide parasitic worms and to confirm anti-inflammatory activity of compounds that has been observed in pre-animal screening tests (mice). In neither case is it possible to complete the work in the absence of animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals employed is the minimum considered to provide information of statistical significance to understanding and designing new strategies for controlling human diseases and is determined in consultation with experienced university statisticians.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The mouse is the chosen species for testing the novel compounds as there are well-characterised, gold standard, industry-employed, models of human diseases that can be utilised with an unparalleled array of reagents and resources to allow elucidation of mechanism of action. The last point is important because the worm product represents a “safe drug” in that although anti-inflammatory, it does not interfere with the ability of the immune system to fight infection. Clearly it is desirable for any drug developed to possess the same property. Also,

	regarding the jird, the presence of the parasitic worm is well tolerated by this species (it is a natural host) with virtually no sign of ill health. All animals are monitored continuously and any showing illness or stress, out with accepted defined levels, treated appropriately in consultation with a veterinary surgeon as required.

Project 23	Modelling and therapeutics for autoimmune disease
Key Words	Autoimmune disease, Gene Therapy, Adenovirus Vector, Autoimmune polyglandular syndrome type 1, Intrathymic injection
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

Viruses modified to remove all their harmful properties are being used for human clinical trials. Our ultimate aim is to develop novel therapeutic agents for diseases of autoimmune disorders (e.g. APS1). To achieve our ultimate goal our strategy is to:

1) Develop novel gene delivery systems

2) Determine if these will alleviate, or reduce disease symptoms.

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)?

1) To develop novel therapeutic delivery systems that would be useful to alleviate, or prevent disease symptoms. 2) In particular to develop new

treatments for APS1 patients using a gene therapy approach 3) These approaches could then be translated to clinic to help treat single gene disorders

What types and approximate numbers of animals do you expect to use and over what period of time?

We will use mouse as the model organism and aim to use no more than 2,000 animals over five years.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

We aim to develop a new technique for the delivery of therapeutic agents that should reduce the pain, suffering, distress or lasting harm that exists for current delivery methods. This approach should result in no more that minor and transient discomfort. The disease that we are working with does however, present with a variety of clinical symptoms that can lead to overall level of severity classed as moderate. All animals will be humanely killed after completion of each study.

Application of the 3Rs

Replacement

We have carefully considered the extent to which these experiments could be replaced by *in vitro* studies using cultured human thymic cells. Thymic cells freshly isolated are then co-cultured with fibroblast cells used as matrix for thymic cells. This system will help us to measure the efficacy and doses of the virus carrying the gene of interest which will potentially give the optimal results in mice. The *in vitro* system will avoid us using animal for the optimisation of the virus doses to be injected to the animal (see reduction section below). The complexity of the thymus microenvironment and limitations of the *in vitro* system would not allow us to fully replace the *in vivo* system.

Reduction

Our general approach is to test hypotheses *in vitro* systems prior to more formal testing in mice. We aim first to test the efficiency of gene transfer vectors in cultured cells and having observed a positive effect, only then we will move on to *in vivo* approaches. Our experimental design optimises the use of animals for data collection e.g. we use the same animals for biochemical and pathological studies where possible. We will continuously monitor our experimental data and refine the design of experiments and the number of animals that might be required to provide statistical relevance. Where necessary we will consult our institution biostatisticians for

confirmation that our approaches use the minimum number of animals necessary.

Refinement

Genetically altered mice and wild type mice are best suited to model the aspects of autoimmune disease that we wish to investigate. We have carefully considered the method of viral vector delivery, one of the options of intrathymic injections is using the ultra-sound probe as guidance for direct non-invasive method of injection instead of more invasive techniques such as a surgery. Such a technique will minimise the harms caused to the animals and the only pain will come from needle stick with injections which is brief and mild and painkillers can be used to mitigate these. At the end of each experiment, animal will be humanely killed. Animal welfare is our priority and we have well-resourced and well-equipped modern animal facilities.

Project 24	**Transplantation of regenerative cellular therapies**	
Key Words (max. 5 words)	Transplantation, stem cells, function, safety, immunogenicity	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Clinical use of stem cell-derived treatments requires the definitive demonstration of their functional efficacy (ie, continue to function effectively in the patient patient), safety (ie, then do not mutate or form tumours in the patient) and immunogenicity (ie, how the patient's immune system recognises and responds to them). The proposed project aims to definitively address these important outstanding questions for a number of key regenerative (stem cell-derived) cellular therapies, including insulin-producing pancreas cells (to treat diabetes), liver cells (to treat liver failure) and platelets (to treat low platelets). Specifically, it aims to determine whether stem cell-derived therapies are effective and safe, and if they are rejected by the immune system.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or	The data generated by this study is essential for conducting human clinical trials using these cellular therapies. It is anticipated that by demonstrating the function, safety and immunogenicity of these cellular	

animals could benefit from the project)?	therapies, their direct translation (in the form of clinical trials) will be made possible. In addition to this anticipated clinical benefit, the knowledge generated will have direct and widespread benefits in the field of regenerative medicine in general, including for the refinement and optimisation of the protocols for the generation of these and other cellular therapies.
What species and approximate numbers of animals do you expect to use over what period of time?	Up to 25,500 mice will be used over a period of 5 years. Up to 875 rats may also be used during this 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 (>90%) of the animals are not expected to show signs of adverse effects that impact materially on their general well-being. No more than 10% of animals are expected to show clinical signs of a moderate severity as a result of the effects of irradiation and immune reconstitution, surgery or treatment with toxin. Very rarely the severity of these signs may be such that the humane end points may be reached. Mice will be killed if they show signs of ill health, such as weight loss, piloerection and hunched posture or inactivity.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The definitive examination of the function, safety, and immunogenicity of stem cell-derived cells and tissues requires examination in intact animals, including those with a competent immune system. This is a necessary and pre-requisite 'final' step for the clinical translation of these regenerative therapies and cannot be completed without animal experiments. Much of the proposed work is carried in the laboratory and using human tissue only, thus minimising the need for animal experimentation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Only regenerative cellular therapies that are supported by compelling in vitro experimental data will be performed using animals. The studies are designed such that many groups of animals will generate valuable data pertaining to the function, safety *and* immunogenicity of the cellular therapies under investigation. Furthermore, some animals

	serve as controls for more than one experimental group, whereas in other experiments, the same animal can be used as its own control (for example, by being transplanted by two sets of cellular therapies). The total number of animals are therefore significantly reduced by addressing all aims using the sophisticated experimental design utilised in this project.
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.	These models used are optimally suited to achieve the aims and objectives of the study. We have refined the protocols and procedures for the generation and maintenance of these mice to maximise the likelihood of the success of the experiments. The vast majority of the experiments are designed such that the animals only experience minor discomfort, and serious ill health or death is never an expected end-point.