



Home Office

NON-TECHNICAL SUMMARY

Potent monoclonal antibodies for biologics discovery

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
 - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Hybridoma, Monoclonal, Antibody, Therapeutic, Transgenic

Animal types

Life stages

Mice

Adult

Rats

Adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

This project licence will be used to immunize animals such as mice and rats to generate antibodies that can be used as therapeutic medicines, as tools to support the development of drugs, as tools for research, and to help improve the methods currently used to discover these medicines and tools.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

We aim to generate new medicines to treat diseases where there is a major unmet medical need primarily in the fields of Oncology (e.g. lung, breast, ovarian, and haematological cancers), Respiratory diseases (e.g. chronic obstructive pulmonary disease, asthma, idiopathic pulmonary fibrosis, and chronic cough), and Cardiovascular Metabolic and Renal disease (e.g. chronic kidney disease, and diabetes), as well as microbial diseases, neuroscience and autoimmunity. The project will also help us discover tools that can accelerate clinical development of drugs as well as help us improve our methods for discovering potent antibodies.

Some of the current treatment modalities for these severe diseases include small molecule chemical drugs that have been found to have severe side effects in human patients. In addition, certain cancers develop resistance to the available therapeutic drugs. Antibodies that are naturally part of the defence mechanism of the human body are thus one of the best options to treat severe diseases with minimal side effects. The antibodies by attaching to the target cancer cell or a diseased cell type can help in the destruction of these unwanted cells from the body. Antibody drugs make up 50% of the current clinical stage drugs and are thus a major class of drugs for the treatment of a wide variety of diseases. As such antibody drugs have the potential not only to improve the outcome of disease for patients but also to cure patients of life-threatening diseases. One such example is an antibody, Panitumumab, that has been used in the treatment of colorectal cancer and has been shown to reduce the disease progression significantly. Another antibody, Imfinzi, that has been used to treat lung cancer has been shown to make significant number of patients better and keep them disease free for several years. In spite of such useful drugs, there is still an unmet need to develop newer treatments because - some of these drugs are only effective in a small cohort of patients or there are diseases where existing treatments are failing and for some diseases multiple combined treatment options are needed. Thus, we aim to generate potent, effective antibody drugs against several such diseases that are able to cure the patients effectively.

What outputs do you think you will see at the end of this project?

- Therapeutic drugs for the treatment of diseases primarily in the areas of cancer, respiratory, inflammatory, heart/lung, kidney, metabolic and neurological diseases
- High quality antibodies to understand the disease mechanism as well as to support the development of drugs.
- Such antibodies will be helpful for machine learning algorithms to learn how to make best quality antibodies. This can ultimately help to replace use of animals for drug discovery.
- Patent applications for projects successful in identifying antibody drug molecule, as well as technology developments arising from these projects.
- Where possible scientific publications and conference presentations highlighting data/learnings from the therapeutic antibody discovery programs. For example, publications directly related to the antibodies themselves, or any further understanding of the disease biology or technological advancement etc.

Who or what will benefit from these outputs, and how?

The potential benefit to patients is enormous, if the projects are successful in generating therapeutic antibodies that are useful for even a subset of patients suffering with diseases of high unmet medical need in Oncology (e.g. Lung cancer – 1.8 million new patients/year, Breast cancer - ~ 2.1 million new patients/year), Cardiovascular Metabolic and Renal diseases (which account for 20 million deaths/year), and in Respiratory diseases (e.g. Chronic Obstructive Pulmonary disease – 384 million sufferers worldwide, Asthma – 339 million sufferers worldwide).

Any new drug development can take up to 10-12 years before it can be used on human patients. Hence, the benefits from this project are long term and may not be fully realised until the completion of the project. At the beginning of each study, we identify a target drug profile e.g. required potency, specificity which tells us about the criteria of a successful drug for that particular disease. Once a panel of antibodies is identified fitting this criteria, we further ensure thorough analysis of these molecules in terms of their physical properties, suitability for manufacturing and a computer simulation based analysis on their dosage in humans. These studies help us identify the right antibody molecule that is likely to succeed through the clinical development. In this way, we ensure the successful onward development of the antibodies.

As mentioned, we also aim to generate tool antibodies that can help support the development of therapeutic drug antibodies. These tool antibodies are required to detect the therapeutic drug antibody or to detect the anti-drug antibodies in human patient samples in clinical studies. There are human drug antibodies which do not show equivalent functionality in animal models. Thus it becomes difficult to study such drug molecules in pre-clinical studies. In such cases, parallel reagent antibodies are generated having similar properties like the drug molecule but showing functionality in the animal models. This helps in the progression of drug antibodies through the pre-clinical study models. Such tool or parallel reagents are routinely required at relatively short notice. Hence, the benefits coming out from such studies are likely to be realised within the lifetime of the license.

Once an antibody is patented after discovery (~18 months) the data can be published or presented. This is likely to happen within the lifetime of the license. This can benefit a wider scientific community

in terms of dissemination of data, and information relating the antibodies and other reagents generated in the form of publications, patent applications, conference presentations/proceedings etc. Where possible antibodies and other reagents generated for each project may be used with external academic collaborators to help further understand target biology and/or develop novel technologies to improve scientific methodologies e.g. we are using antibodies and their hybridoma cell lines to investigate and develop technologies to improve antibody isolation using microfluidic techniques.

How will you look to maximise the outputs of this work?

We will look to publish manuscripts and patents, as well as present our work at conferences regarding the discovery of new drugs, tool reagents and developments of new methods as well as novel insights into the biology of diseases. We will also consider publishing unsuccessful approaches and non-significant data via open access platforms such as F1000Research.

Species and numbers of animals expected to be used

- Mice: 2300
- Rats: 50

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Small animals such as mice and rats are the animal species of choice for antibody isolation because they are easily handled and easy to care for. The immune response in these animals is well understood, is generally robust and reproducible. Immune systems of mice and rats are similar to human immune system that help generate large and diverse types of potent antibodies. Furthermore, *in vitro* (in laboratory) isolation of antibody genes from the cells of these animals to help generate antibodies on large scale in laboratories is well understood. Adult animals (typically 6-8 weeks old, not exceeding 6 months of age) of either sex (male / female) are typically used for generating antibodies, as the immune response is immature at an early age and weakens in older age. Certain human disease targets can have equivalent (homologous), functional counterparts in mice. Hence, it is difficult to generate an immune response to such targets in mice. In those cases, we would use rats to generate antibodies.

Typically, what will be done to an animal used in your project?

Typically, at the start of a protocol, animals of appropriate age will be labelled (to identify individual animals), weighed, and blood sample will be collected as a pre-immune sample. Depending upon the protocol, animals will be injected with an antigen (substances such as proteins/RNA/DNA/cells against which antibodies are generated) via suitable routes such as subcutaneous (under the skin), intra peritoneal (in the stomach cavity) etc., multiple times over the duration of the protocol e.g. 28 days.

Animals will be regularly monitored for characteristics such as weight, behaviour, appearance in between the injections.

During the in vivo (performed in or on living animals) experiment, blood sample will be taken to test the generation of an antigen specific antibody response. There may be a small number of studies where we would be interested in imaging the animals in a non-invasive way to check the expression of a target antigen upon genetic immunization (using RNA / DNA). This will enable us to develop and optimise this immunization strategy, which has the promise to improve immune response to challenging drug targets (complex targets on the cell surface), which are a major class of therapeutic target (~30% of all marketed drugs).

At the end of the protocol, animals will be humanely killed and organs such as spleen, lymph nodes and bone marrow will be harvested. The B cells (cells secreting antibodies) from the harvested organs will be used to discover potent antibodies against the target antigen using established laboratory methods.

What are the expected impacts and/or adverse effects for the animals during your project?

100% of the animals are likely to experience mild levels of severity such as transient discomfort/irritation at the site of injection, transient weight loss. This is because they will undergo repeated administration of substances and sampling using standard routes. Unless otherwise specified, the administration of substances and withdrawal of body fluids will be undertaken using a combination of volumes, routes and frequencies that of themselves will result in no more than transient discomfort and no lasting harm. Animals are not expected to show any deviation from normal health and behaviour. The mentioned adverse effects are expected to last only for a short duration and can be managed by, for example, administration of food enrichments. We have observed a pattern of animals showing weight loss 24h-48h after the immunization without any apparent clinical signs. At this point animals are provided with food supplements. The weights start to recover 24-48h post onset of the weight loss. Any animal that does not recover the weight loss within 48h will be culled immediately. Any animal showing clinical signs in addition to the weight loss will be culled immediately. Animals showing weight loss of more than 15% will be culled immediately. Based on our experience on previous licenses, few incidences of transient anaphylactoid responses may be observed after the last injection that do not cause long lasting harms. The symptoms might include one or more of the following: swollen snout and/or paws, piloerection (erection of hair), hunched posture, inactivity but responsive. These symptoms typically manifest within 2 hours post-immunization. Such animals will be monitored closely. These symptoms usually resolve without any intervention within 2 hours of onset. If no sign of improvement is observed over the observation period the animal will be humanely killed. In case of a severe anaphylactoid reaction, such as seizures typically occurring within 15 minutes of dosing, the animal will be killed immediately.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mice

Mild Severity - 100%

Moderate severity - 0%

Rats

Mild severity - 100%

Moderate severity - 0%

What will happen to animals used in this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The use of animals in this project is required to generate effective antibody medicines and antibody tools which could help treat different types of diseases with high unmet medical need (e.g. cancer, diabetes, asthma). Immune systems of mice and rats have natural capabilities to generate high affinity (a natural property to attach to the target specifically and tightly) antibodies to the target antigen (a substance to which the antibody binds) with the help of processes such as affinity maturation where cells secreting high affinity antibodies are gradually selected over the ones secreting low affinity or non-specific antibodies. Since the antibodies are generated inside a living animal they are naturally selected by the immune system for beneficial characteristics such as specificity to the antigen and absence of any unnatural structural changes. With the use of genetically modified mice expressing human antibody genes, high affinity human antibodies useful for therapeutic purposes can be easily derived.

While there are in vitro (in laboratory) technologies for antibody generation available, these methods are not suitable for all disease targets. For example, it is extremely challenging to generate medicines to disease targets which are found on the surface of cells using in vitro technologies. Antibodies to simple soluble protein targets (not attached to cells) can be generated using in vitro technologies. However, to generate a potent drug out of it, each antibody derived in vitro will need to undergo further in vitro manipulation which is often very long, require great efforts, and typically performed on one antibody at a time. This could result in significant delays in bringing therapeutic drugs to patients in the clinic. Additionally, the process of improvement can have other side effects on the drug making it difficult to manufacture and the potency of the drug cannot be guaranteed.

A review of the most appropriate approach for generating our medicines will be carried out at the beginning of each programme. We will actively search for, sought access to, and interrogate third-party suppliers and libraries to confirm, if the antibody that we desire is already available. We will also apply

the learnings from successful projects carried out on this licence to help build our knowledge of how to make in vitro antibody generation technologies more reliable in future.

Furthermore, we have introduced new technologies to allow us to perform deep searching of B-cells generating potent antibodies directly from immunized mice. This new technology has the power to generate very large antibody data sets which will be used to help train machine learning (use of computers to analyse data and identify patterns) algorithms. The aim of this initiative is to determine if we can train machine learning algorithms to design high affinity antibodies in silico (using computer simulation) to ultimately replace both in vivo and in vitro antibody discovery technologies. The datasets generated from in vivo studies are especially valuable, as these antibodies have been generated naturally to the target antigen, and then affinity matured by natural processes. Currently, there is no in vitro system that can be used to model the complex antibody generation process that the in vivo antibody generation and affinity maturation provides.

There has been significant scientific discussion about the justifiable use of animals for therapeutic biologic drug discovery after EURL ECVAM recommendation on non-animal derived antibodies (European Commission, Joint Research Centre, Barroso, J et al 2020 <https://data.europa.eu/doi/10.2760/80554>). However, it has been clarified separately by few of the original authors on this report, that these recommendations for non-animal derived antibodies were made excluding therapeutic antibody discovery and development (Bradbury et al, MAbs 2021). The authors also further opine that currently there are significant challenges in widespread adoption of in vitro platforms that could compete with / replace the in vivo biologic discovery process. Until such challenges are overcome, it is important to keep both options in the future for therapeutic antibody discovery (Bradbury et al, MAbs 2021). In addition, European Animal Research Association (EARA) and the European Federation of Pharmaceutical Industries and Associations (EFPIA) along with others have also called these recommendations as premature highlighting that – animal derived antibodies are critical tools from basic research to development of life saving drugs, non-animal derived antibodies cannot recapitulate all the useful properties of animal derived antibodies and as evident recently from COVID-19 research, animal derived antibodies play key role in development and large scale production of therapeutics (<https://www.eara.eu/post/eara-efpia-response-to-antibody-recommendation>). Thus, it is significantly valuable to generate potent antibody drugs by immunizing animals which could help treat millions of patients worldwide.

We have also considered the recommendations made in the report by Lord Sharpe: 'Review of antibody licences: letter from Lord Sharpe'. We have made attempts to thoroughly review our 3R strategy accordingly.

Which non-animal alternatives did you consider for use in this project?

We have access to in vitro antibody discovery technologies such as phage display, a technology where antibodies are displayed on the surface of a virus particle. Before starting a new immunization campaign a full scientific review of the target antigen and antibody requirements will be carried out which will enable us to choose the most appropriate antibody generation platform (i.e. in vivo vs in vitro) for each target. There will be target types and specific requirements where phage display may be the only route to generate antibodies e.g. where there is a high degree of similarity between human and mouse antigens, a high affinity antibody is not required and/or where a very specific region of a

protein is required to be targeted where in vitro display technologies would have an advantage over an in vivo approach.

Both in vivo and in vitro derived antibodies / datasets will help develop our machine learning and in silico antibody discovery platforms, which has a potential to replace both the discovery platforms in the future.

We are also aware of the NC3Rs website and database which we will access regularly to receive latest updates on non-animal alternatives.

We would also plan to engage AWERB on selecting non-animal derivatives, by presenting our findings/ progress with the committee regularly. In addition, we can discuss some of our up coming projects and discuss our justification for the use of animals. We can also share any novel non-animal technologies with AWERB forum to receive feedback on non-animal derivatives in our projects.

Why were they not suitable?

In vitro antibody discovery technology is likely to generate artificial antibodies which may not exist naturally in the human body. This can give rise to undesirable properties in those drug molecules which are identified later on during their development process. Improving upon these unwanted properties in a drug can delay the development of medicines for unmet needs. Currently, there is no in vitro system that can be used to model the complex antibody generation process that the in vivo antibody generation and affinity maturation provides. It is known that isolating functional antibodies to targets that are expressed on cell surface is challenging for in vitro technologies. Where large numbers of high affinity antibodies are required as drugs or as tools, the restricted use of in vitro antibody generation methods would result in significant delays in the progression of drug molecules into clinical trials in humans by years which, for the major diseases which we aim to tackle such as cancer, diabetes, asthma, etc., could potentially result in many more patients dying from such diseases than is necessary.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of animals used per project will be based on the aims for the particular study, for example, whether the requirement is a drug or a tool antibody, mode of action required (e.g. binder to target, neutralizing a target etc.), our previous experience with a similar project, available knowledge and the likelihood of success. Therefore, the number of animals used per project will vary with the target type and aim of the project. Based on the previous license experience, as well as scientific research and consultation with experts within the company, 3 groups each consisting of up to 6 animals is generally

sufficient to obtain the required results for experiments involving therapeutic antibody discovery. However, for certain targets, based on prior experience, groups sizes as low as 3 animals per group can be used to generate a successful outcome for the given project. We will also explore the possibilities of combining pilot studies for new studies along with our studies to refine our procedures resulting in usage of fewer number of animals. We have set a conservative figure of 2300 mice over the 5-year licence period. This is with the assumption of doing 100 drug discovery projects and 25 tool discovery projects over the span of 5 years.

Rats are occasionally used in the generation of tool antibody reagents, that help in the development of human drug molecules. For rat studies, we use 3 rats per group, up to three groups per study. For our previous licence, no projects have required rat immunizations to date. However, we would like to retain rats as an option should the need arise and estimate 50 rats would be used over the course of this licence. As a demand led PPL, it is difficult to predict total animal usage.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We prepare a statistical health check report for our studies that includes details about the study design such as study groups, planned data analyses, assumption of the analyses, justification of animal numbers and how we control the bias and variation.

In vivo protocols are designed to use minimum number of animals to achieve study objectives. Our immunization protocol allows us to generate strong antibody responses over the shortest immunization time possible, helping us in efficient use of number of animals. We used our experience from previous experiments conducted on our past licences over the past 15 years, as well as literature searches and consulted with experts within the company to guide the design of our studies where appropriate.

I am familiar with the online tools available for help with the experimental study design such as NC3R's Experimental Design Assistant. I have also read literature on designing experiments with appropriate sample size on FRAME (Fund for Replacement of Animals in Medical Experiments) website: <https://frame.org.uk/resources/experimental-design/>. The availability of expertise and the literature/online resources have helped guide the experimental design.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will use our experience from previous experiments, literature searches and consultation with experts within the company to guide the design of our studies where appropriate. When we are investigating a new method of antibody discovery, where possible we will combine these activities with real drug discovery projects in order to minimise the number of mice used, whilst still giving us a comparator groups of standard best practice. Where appropriate, small pilot studies will be conducted to ensure that the study objectives can be met. We will adopt a fit-for-purpose immunization strategy where possible to make the best use of animals. For example, based on our previous experience, it is hard to generate a strong, uniform immune response in certain transgenic mouse strains following a typical immunization protocol. This requires using more number of animals to obtain the required antibody outputs. Thus, specifically adapting the immunization regime in such cases in terms of increasing the duration of immunization and the number of doses will allow us to generate a stronger

immune response thereby successful generation of antibodies. We have developed a new method where we can screen antibody secreting B-cells directly for antigen binding. We are also working on cutting edge platforms where we aim to screen B cells directly for functional antibodies. Thus, employing multiple antibody discovery platforms will help us maximise the output from the immunized animals and eventually reduce the number of animals required.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Mice and rats are small, easily handled species with highly characterised immune systems and well-defined biology. Mice and rats are short lived, have rapid generation times, and are easier to look after than other larger animals. We plan to use our proprietary transgenic mouse strains and other commercial strains (e.g. Ablexis) to generate fully human antibodies. We also plan to use wildtype mouse strains such as CD-1, NZB as well as wildtype rat strains. For studies requiring generation of fully human therapeutic antibodies we use transgenic mice whereas for tool / reagent generation studies, we use wildtype strains. Hybridoma technology that generates immortal antibody secreting cells in laboratories using the cells from animals is routinely used for antibody discovery. The animals are kept in high quality pathogen free facilities by highly trained animal care staff to ensure that the animal welfare is paramount. Animals are given environmental enrichments and will whenever possible be group housed.

The antibody response in mice is well understood. The injection routes used in this project have all been shown to cause no adverse effects (i.e. Mild severity) whilst inducing effective antibody responses in the majority cases.

Rats tend to be used when the target antigen is mouse or similar to mouse. Because it is difficult to generate an immune response to a 'self like' substances in animal bodies.

Our immunization protocol allows us to generate high quality, high affinity antibodies over the shortest period while maintaining a high level of animal welfare. Our immunization protocol is relatively short in duration that helps the antibody secreting B-cells to be predominantly located in specific small organs called lymph nodes. Longer immunization protocols would result in B-cells migrating to other organs making it difficult to isolate such desirable B cells. Shorter immunization protocols are described in the literature, however, these shorter protocols involve far more immunizations per time point and are likely to have a significant impact on animal welfare, therefore, we do not use these.

Why can't you use animals that are less sentient?

Mouse / rat immune system is well studied and there is a significant knowledge available in the literature about various routes, procedures, and outcomes with respect to immunizations. Based on the scientific data and our previous experience, we intend to use adult animals of either sex, typically 6-8 weeks old, not exceeding 6 months of age. The animals of adult stage have an active, fully functional, mature immune system as compared to being either immature at an early stage or weak as the animals get older. Hence, we prefer to use animals of adult stages.

To generate antibodies, animals are injected multiple times with the antigen. This process takes advantage of the natural mechanism of the immune system where repeated exposure to the antigen allows for generation of potent, high affinity antibodies. Thus, it is required to use animals of adult stages which can be efficiently immunized over a period of few weeks.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We have been working to refine our immunization practices, as mentioned in the previous PPL licence. Using our original immunization protocol where mice were immunized sub-cutaneously while awake, we found antibody responses to our targets were variable within a group of mice despite all receiving the same dose of target. Furthermore, we saw an incidence of severe immune reaction (anaphylactoid) which, when it occurred, often resulted in the mice having to be culled as they did not recover within agreed timeframes for this mild severity licence, or in extreme cases the mice died shortly after the injection. This incidence rate was calculated to be around 2% year on year.

We have observed a pattern of animals showing weight loss 24-48h post an immunization. There will be regular monitoring of the animals 24-48h post immunization and increased monitoring subsequently, as required. In addition, post onset of body weight loss, food supplements will be provided.

Our revised procedure now involves administering the sub-cutaneous injection while the animals are under general anaesthetic (a substance that induces insensitivity to pain). This is much easier for the in vivo staff to administer, and placement of the needle and the injected material is far more accurate. The result of this change has meant that we now have a far more even antibody response to our targets within a group of mice. This has enabled us to reduce mouse numbers per group on many projects. Furthermore, the incidence of severe reactions to immunization has decreased significantly, whereby very few mice have had to be culled or have died during the procedure. In addition, whenever required we aim to ameliorate animal harms by providing nutritious food supplements, regulated temperature and analgesics (substances that relieve pain).

We will investigate further, to see whether we can ultimately reduce the number of immunizations given to each mouse per project. We will also investigate whether giving the final boost sub-cutaneously can help to reduce the incidence of any anaphylactoid like effects occasionally observed at the final dose with intra-peritoneal immunizations.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will adhere to the published best practice guidelines from LASA to conduct our experiments. We will also follow ARRIVE guidelines to ensure in vivo experiments are planned and carried out in the

most robust and reliable manner. We are also aware of the PREPARE guidelines, which will be used to increase the reproducibility and quality of our research.

Our study on antibody generation requires us to use small adult rodents which have mature immune system and thus are able to provide us with potent antibody drugs. Currently, there is no animal model available that is less sentient than rodents and could provide potent antibody drugs for the life threatening diseases that we aim to counter.

We are also committed to the ethical use and welfare of animals. Learnings from the previous PPL license will help us conduct our experiments in the most refined way.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will keep up to date about the advances in the 3Rs by regularly accessing the online resources such as NC3Rs and FRAME. Before starting a new animal immunization campaign, we will carefully review the pros and cons of such an exercise as well as thoroughly analyse whether an alternative in vitro methodology can be used as a replacement. This will help us achieve our 3R aims. We also aim to explore new technologies for antibody discovery and seek to reduce the animal usage as well as refine our techniques.