Home Office

NON-TECHNICAL SUMMARY

Human antibody generation platform

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Human antibody, Immunotherapy, Immunisation, Ion channels, Membrane proteins

Animal types	Life stages
Mice	adult, juvenile, embryo, neonate, pregnant

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?

The objective of this project is to enable the discovery and development of novel fully human antibody therapeutic agents. Immunotherapy has had a significant impact on treating cancer and we believe that antibodies can also have a benefit on treating other therapeutic areas such as inflammation, infection, pain, and neurodegenerative diseases.

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.

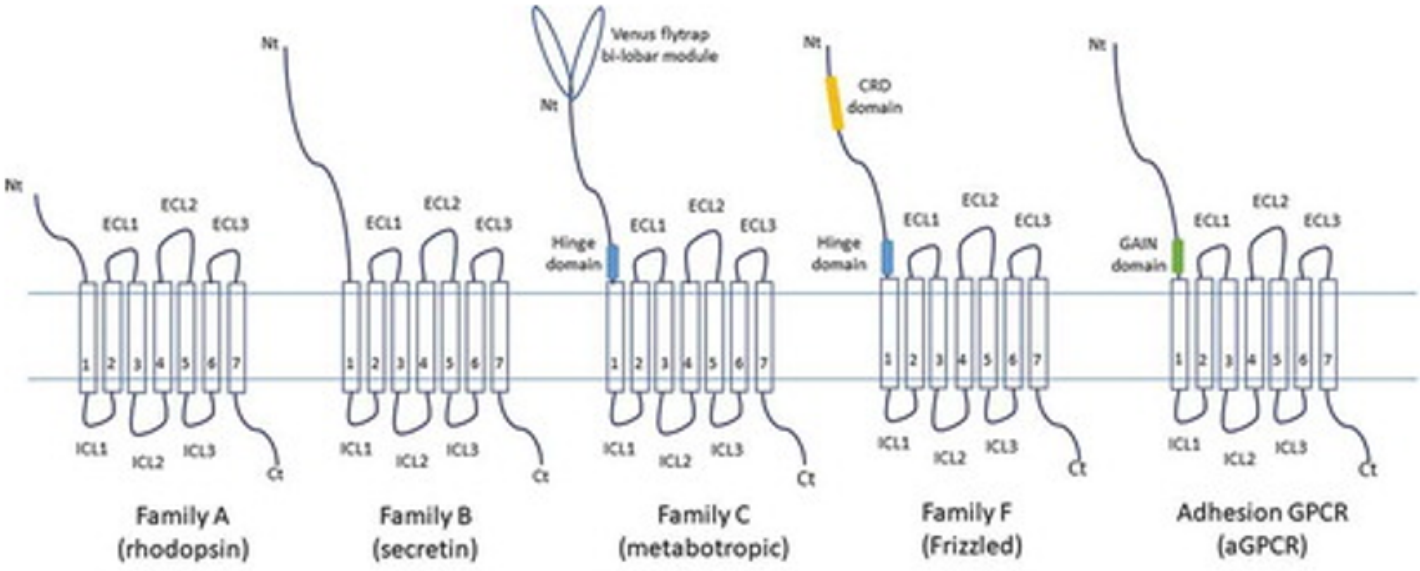
Why is it important to undertake this work?

This project will be using mice expressing fully human antibodies which could be used as therapeutics without any further modifications. Because antibodies obtained from these transgenic mice are fully human, they have already been optimised through the immunisation process, and can be used as such as therapeutics in humans.

This discovery technology-driven project will be focussing on diseases involving targets for which no antibody exists because of the structural complexity of the proteins involved which cannot be produced in cell culture. The collaborative approach to generate data for this project will allow us to identify novel biological drugs and to develop new ways using monoclonal antibodies to treat patients with incurable diseases and conditions such as neurodegenerative diseases, chronic pain, or cancer.

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

The project will be focussing on targets for which no antibody has been generated yet. For example, Ion channels and G-Protein coupled receptors (GPCR) are protein membranes for which only small molecules have been developed.



These proteins are involved in all therapeutic areas including cancer, inflammation, pain, auto-immune, and neurodegenerative diseases. The output of the project will be to deliver novel fully human therapeutic antibodies with new mechanism of action against this target family. So far only few

antibodies have been approved in immunotherapies, such as Mogamulizumab, to treat T-cells leukaemia. Nevertheless, Mogamulizumab is an antibody that was identified from the immunization of mice with a CCR4 peptide exposed on the membrane as the full protein could not be used for immunisation.

This project will obtain antibodies against the whole protein instead of a peptide and there will be no need to make those antibodies human as they did for Mogamulizumab because our mice will produce human antibodies already.

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

The potential benefits of this project will be the progression of new therapies into clinical development and ultimately onto the market bringing benefit to patients. We intend to develop between two to four antibody candidates for therapies such as cancer, pain, auto-immune diseases, or inflammation into pre-clinical studies over the next 5 years.

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

This is a technology discovery licence, projects are done in collaboration. The project output is fully human antibody. According to published literature and available technology, that is the fastest way to generate a new human therapeutic. However, it is important that each output is fully utilised and developed into the clinic. The partnerability of each asset will be a key criterion when selecting the right target. It is important that we make sure that there is an interest in the field to develop such a drug before we engage in an immunisation campaign.

Species and numbers of animals expected to be used

- Mice: 4000

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.

Rodents, and especially mice, are very easy to manipulate genetically and as a result have been engineered to produce chimeric mouse/human or fully human antibodies. These mice have been genetically engineered to produce human antibodies after immunisation with an agent which is different enough from a mouse protein to trigger an immune response. Mice will be used as adult with a fully developed immune system.

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

Because of the project prioritising complex proteins as agent to obtain antibody against, different immunisation protocols have to be used to maximise the chance of success. Standard protein immunisation regime will be used combining purified antigen mixed with an adjuvant to stimulate the immune response. Proteins will also be expressed in mouse cells to keep their biological functions and cells will be directly injected in the mouse abdomen to stimulate immune response. And finally, DNA encoding for the protein of interest will be injected to mice using two different methods (Tail vein delivery and DNA tattooing), those two methods will be performed under general anaesthesia. Depending on the target type, specific method will be prioritised to reduce the number of animals used.

Over the course of the immunisation, several boosts (up to 6 depending on the method used), will be performed. Antibody levels will be assessed by regular blood sampling. if the antibodies are present and in high enough numbers (high titre), mice will be humanely killed and immune tissues (spleen, lymph nodes, and bone marrow) will be removed. B-cells which are reactive to the protein of interest will be isolated from the immune tissues, mRNA extracted, and genes corresponding to the antibody will be sequenced. No more animals will be used in that experiment.

After a successful antibody discovery campaign, pharmacokinetics (PK) of the human antibody will be assessed by injecting the antibody intravenously to transgenic mice expressing human neonatal Fc receptor by measuring the level of antibody over time by taking a drop of blood sample. This will provide valuable information whether the antibody can be developed as human therapeutics.

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

The mice are not expected to have any severe adverse effects from immunisation. Antigen and whole cells immunisations can occasionally lead to mild symptoms such as local swelling or non-lasting inflammation at injection site. If any adverse effect exceeds the mild category or is not temporary, the animal will be humanely killed. At the end of an immunisation schedule, all mice are humanely killed.

When raising antibodies against proteins expressed using DNA immunisation, special technology has been developed to do so. This technology involves the use of DNA coated gold particles which are introduced to the animal via bombardment of the skin with pressurised gas (Gene gun intradermal injection). This procedure is carried out under general anaesthesia and has minimal associated effects, which can include slight redness of the skin at the site of inoculation.

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)?

Some adjuvants which are very effective in stimulating an immune response can cause tissue reactions in the animals at the site of injection, therefore the use of these adjuvants is carefully controlled, with any reaction being closely monitored. Subsequent blood samples will be taken from an animal in order to test the level of antibodies being produced within the animal. These blood samples will be taken from an appropriate collection site on the animal such as veins/arteries and as such can (but rarely) lead to the formation of bruising and slight skin damage. The level of severity will be mild for most animals.

For the Hydrodynamic tail vein injection, performed under general anaesthesia, 10% animal weight (~2 ml) of a constant concentration of DNA is injected, and the animal is expected to recover within 30 minutes without any side effect. Those two procedures have a moderate severity.

Upon reaching a desired level of circulating antibody against the antigen of interest, an animal will be given terminal anaesthesia, their blood will be collected and antibodies tested. When this has been done, immune tissues (spleen, lymph nodes) will be collected for further scientific use. Although significant adverse signs within any animal used for the production of antibodies are not expected full veterinary attention will be provided should there be any unexpected consequences of any procedure carried out. All animals used for the production of antibodies under the authority of this licence are subject to well defined humane endpoints, which, if experienced, will result in the animal being removed immediately from the study.

What will happen to animals at the end of 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 overall aim of the project is to discover new human antibodies towards difficult targets by using specifically engineered strains of mice able to produce human antibodies. The animals used in this licence will be used to develop new antibody therapies to treat conditions involving targets which are difficult to express in solution and for which methods like phage display are not applicable.

Also, ensuring that potential medicines with suitable pharmacokinetic – (what the body does to the drug (PK) properties can be selected for further development to treat human disease effectively. PK is investigated by studying how potential medicines are absorbed and distributed in the body as well as how they are broken down (metabolised) and excreted.

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

Phage display using fully human universal libraries is the closest method to replace human antibody transgenics animal immunisation and we are using this method currently for projects whose targets can be expressed in solution. This project is mainly focused on therapeutic indications for which recombinant proteins are not stably expressed in solution hence phage panning cannot be performed. Those targets (i.e. Ion channels or G-protein coupled receptors) form an integral part of the membrane and need to be expressed in vivo by using whole cells or DNA immunisation by making animals to produce the target in their cells to trigger an immune response.

Why were they not suitable?

The purpose of using humanised mouse strains to produce human antibodies is that they will use the complete human repertoire of possible antibodies whereas a phage display library will have constraints on the human framework and on the size of the target-recognising loops (CDRs) of the antibody. The complete human repertoire antibody coverage will allow the immunisation process to discover highly potent antibodies which could be ready as therapeutics whereas the potency of antibodies obtained by phage display is limited in potency and will necessitate further lengthy affinity maturation process.

From experience, although the phage panning process is trying to mimic the physiological binding, the conditions the target is attached to a plastic matrix make some portion of the target not visible to the phage presentation and will influence the selection of non-specific binders (i.e. plastic-binder) which will need to be deselected in the process. Some key epitopes could then be masked and antibodies targeting those hidden motifs will be lost. It is therefore important for these targets to be expressed on cells (for cells immunisation) in vitro or in vivo (with DNA immunisation) so they keep their functional structure and trigger specific immune response.

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 aim is to run up to 10 projects per year knowing that each immunisation project will use 30 mice (10 mice per protocol) based on the recommendations of the mice developer who has done this for over 10 years. 300 mice will be needed per year and 1500 for the 5 years of the licence for immunisation. Half of the projects will require knock out generation because the percentage identity between the human target and its mouse equivalent will be over 85%. 20 wild-type females mice and 2 males will be required for this work per project, therefore ~100 per year and 500 for the duration of the licence. We estimated that half of the mice obtained from the breeding will not be required for projects because of male's redundancy (mostly females are required for knock out generation). We will therefore prioritise males for immunisation projects. So, if 2000 mice are required for project work or knock out generation, we estimated that 4000 mice will be required for breeding and project work. Animals not used in projects can used in collaborative research.

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

For conventional antigen immunisation of standard wild type mice, 5 mice will be enough to generate successful discovery of antibodies. As we will be focussed on difficult targets to be expressed by mouse cells, we will be using three different immunisation methods and increasing the number of mouse per protocol to 10 to be sure that antibody titres will be optimal for at least 5 mice. This recommendation comes from the mouse developer.

Although we will use the mouse developer recommendations for the first few projects, the aim is to select the best protocol for each target and to decrease the number of animals used per protocol if possible. Ideally we should be in a position after several projects to limit the usage of mouse for immunisation but also to select what is the best protocol per target type to increase chance of success with the aim of decreasing the number of mice used per project by half at least.

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

The first few projects will dictate the success rate per protocol and we will ask the question whether this success could have been obtained with less animals and if an immunisation method is better than the others. We will then implement the result and learnings to subsequent projects so we reach a steady-state whereby we are confident that the least number of animals and methods are used for maximum success.

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.

The mouse strains used in this project have been specifically engineered to produce human antibodies. Therefore, outputs from this project consist of potential therapeutics ready to enter the clinic without further modifications. Those genetically-altered animals can only be used for this type of approach and are dedicated to human antibody discovery.

Immunisation methods used are mostly mild and will not cause any lasting harm. Antigen and cells immunisation will be performed sub-cutaneously and intra-peritoneally without need of analgesia. DNA immunisations will be performed under general anaesthesia for the ease of the procedure and the safety and wellbeing of the animals.

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

For immunisation to be successful, we need adult with immune system fully developed. Mice are best organism for this work as they present similar immune response to humans.

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

Experimental procedures may involve a limited number of injections and/or small blood samples (the latter using local analgesic cream) over a period of several weeks. These will be conducted according

to best practice guidelines by trained and competent staff. Most procedures will be classed of being of Mild severity and have only a transient impact on the animal. Any concerns regarding the health or welfare of an animal will be discussed with the Named Veterinary Surgeon or the humane killing of the animal. At the end of the procedures, animals will be killed using a recognised humane method detailed in Schedule 1. After every experiment we critically appraise what we do to seek out any ways to improve our models to reduce harm to animals. This strategy has been highly successful and our models continue to show improvement in this area.

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

NC3Rs, NORINA, and http://www.procedureswithcare.org.uk/ provide a list, database, and videos of best practices and alternatives to using animals and refinement solutions such as ARRIVE Guidelines and PREPARE (norecopa - https://norecopa.no/prepare)

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

Regular interactions with the establishment named persons will allow us to be aware of the latest innovations in the field of the 3Rs but we will also receive regular newsletters from key 3R organisations such as NC3Rs or ALTWEB, and Best practice guidelines on Antibody production.