Home Office

NON-TECHNICAL SUMMARY

Developing new multivalent recombinant vaccines platforms against infectious diseases.

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
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Vaccine, Infection, Prevention, Immunology

Animal types	Life stages
Mice	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?

The overall aim of the research is to develop new and improved vaccines. These will be low cost vaccines that can protect against several disease that affect children and adults in Low-Middle-Income countries (LMIC) and in the Western world.

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?

There is an urgent need for improved vaccines against infectious diseases. New vaccines need to be safe, well tolerated, effective and offer broad coverage against many pathogens. In fact, multiple pathogens often coexist in the same geographical areas and a single vaccine able to control these diseases would be a desirable asset.

There is also an urgent need to make vaccines that are easy to develop and produce at low cost, to benefit poorer countries via increased affordability.

It is important to develop vaccines that are based on simple construction and therefore suitable to move from early experimentation to production in the shortest possible time. The design and realisation of new vaccines should be simple enough to allow modifications that increase protection against multiple pathogens in response to epidemiological changes and also to allow preparedness for the potential emergence of new and unknown diseases with pandemic potential.

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

The main output of the project will be the generation of new, safe, effective, versatile, vaccines against bacteria that cause grave infections worldwide in adults and children. The vaccines will protect simultaneously against many variants of each bacterial pathogen, given that these variants often coexist in the same geographical area or circulate between different areas, emerging suddenly and unpredictably. The project will yield vaccines mainly, but not exclusively, against bacteria that cause acute infections for which antibiotic treatment often fails. The project will target mainly bacterial meningitis, gonorrhoea, salmonellosis and can be extended to other infections caused by bacteria, virus and parasites.

The information produced by the project will be divulged in due course for the benefit of the scientific community. This will happen in the form of scientific publications and presentations at meetings.

The work will produce intellectual property that will support in the future grant and patent application and clinical trials.

We will also engage with the general public, via the University press office, the engagement channels of our industrial collaborators, via science festivals and informal public engagement talks and via appropriate contacts with the press and the media.

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

The project will primarily benefit adults and children at risk of acute and chronic infections caused by meningococcus, gonococcus and *Salmonella*. These diseases are widespread globally with an unpredictable epidemiology and devastating epidemic potential especially in Low and Middle Income countries of Africa and East Asia. These diseases have a very high level of resistance to antibiotics and therefore their prevention through large-scale vaccination programmes remains the best option at a global level.

The short-term output (3-5 years from commencement of the work) will be the construction, development and testing at the preclinical level (to the end of animal experimentation and ready to enter studies in humans) of candidate vaccines able to protect against multiple variants of each one of these pathogens.

This will be followed by the necessary steps for the production of clinical batches of the vaccines, licensure and organisation of clinical trials in humans (5-6 years from commencement of the work). Trials in humans will follow and will be completed within 6-10 years from commencement of the work.

Given the recent global experience with vaccines against COVID-19, and the current collaborations that we have with industrial partners, these timelines may be shortened to deliver the vaccines.

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

We collaborate with other academic institutions and with industrial partners. This will allow us to expedite the research phases of our work and also to perform the essential steps required by the future development, testing and production of the vaccines. Therefore, the outputs of this research will be impactful. We will divulge our work via open access publications and talks at conferences subject to prior protection of intellectual property if required. We will engage with the public to share and explain our research via open days, interaction with the media and suitable press releases from the University.

Species and numbers of animals expected to be used

- Mice: 5000

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.

We shall use adult mice to test the immunogenicity of the vaccines. The mouse model is a reliable and broadly used small animal model for the initial evaluation of immune responses and protection induced by vaccines against many bacterial pathogens. Results obtained in mice have informed vaccine development against many pathogens of humans and other domestic animals for decades.

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

Vaccines will be injected into animals. They may be injected intravenously (*i.e.* into a vein), intraperitoneally (*i.e*. into a body cavity), subcutaneously (*i.e.* under the skin), intranasally (*i.e.* instillation of small volumes into the nostrils), orally (*i.e.* into the mouth or stomach). Booster doses may be given at 2-4 weeks intervals, usually for a maximum of 4 doses, to mimic the number of doses that will be given to humans if the vaccines were successful in the later clinical phases.

Blood samples will be taken after each vaccination to test for the induction of immune responses.

In some cases, animals will also be injected with drugs that act on the bacteria (*e.g.* antibiotics) or that increase immunity (*e.g.* antibodies).

In some cases, vaccinated animals and a small number of unvaccinated control animals will be infected for a short time with low, subclinical, doses of live bacteria.

At the end of the experiments the animals will be humanely killed.

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

The vaccines will consist mainly of inactive preparations and therefore cannot cause infections. In some cases we may use live bacteria that are genetically impaired in their ability to grow in a mammalian host. The vaccines will have been constructed and previously tested in non-animal models (*e.g.* cell culture, chemical tests etc.) to make sure that they are likely to have the lowest possible level of side effects.

Our experience on immunity to bacteria and vaccine development, acquired during several decades of fundamental research, allows us to predict correlations between the type of immune responses induced by vaccines and the presence or absence of protection. In the case of *Neisseria* vaccines, serological tests (*i.e.* serum bactericidal activity, SBA) can be taken as predictors of vaccine efficacy. Therefore, only in rare cases we will need to re-infect vaccinated animals with virulent pathogens to conclusively determine vaccine efficacy. This will happen, within this project, in the case of *Salmonella* infections where a firm and conclusive immunological correlate of protection is not available. When this is done, the infection experiments with live bacteria will be performed at low doses and their duration will be short, thus minimising clinical signs, should the vaccine prove not to be sufficiently efficacious.

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=96%

Moderate=4%

Severe= 0%

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?

Animals are needed to test the quantity and types of immune responses induced by the vaccines that we are currently constructing. In some cases, we also need to infect animals with low doses of live bacteria to ensure that vaccines can protect animals against disease. This is the necessary prelude to human testing in clinical trials.

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

There are no non-animal alternatives to conclusively test the immunogenicity and protection of vaccines. These need to be ultimately tested within the complexity of the immune system of a whole mammalian organism.

We routinely use non-animal alternatives to test the side effects of our vaccine preparations, to determine their chemical composition, measure the presence and quantity protective components within the vaccine. This is done before any animal experimentation takes place.

We use cells in culture, chemical tests that measure the composition of the vaccine and immunological tests that predict the efficacy immune responses to the vaccine. For example, in the case of *Neisseria* vaccines, we measure the ability of animal sera to kill bacteria as an indirect measure of vaccine activity and protection.

Why were they not suitable?

Because it is impossible to evaluate if a vaccine stimulates immune responses unless a whole animal is injected with a vaccine. Non-animal tests (see above) can in some cases be used to measure the presence of immune responses induced by the vaccine in the animal.

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?

We have 30 years of experience in designing animal experiments with the aim of testing vaccines against bacterial infections.

We estimated the numbers of animals according to the expected number of vaccines that we shall test, considering groups sizes and experimental protocols. The numbers of animals within each experimental group and the size of each experimental group are decided as indicated in the sections below. Briefly, throughout the project we estimate to test approximately 200 possible candidate vaccines in small-scale experiments involving 10 mice per candidate on average. We expect to take forwards about 10% of these (20 candidates) for further immunogenicity and functional studies that will involve approximately 150 mice per candidate vaccine.

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

We design experiments to include as many experimental groups in parallel as possible, with the smallest number of animals per group (as determined by statistics, pilot experiments and mathematical modelling); we associate one control group to as many experimental groups as possible so to reduce the number of control animals used in our experiments. We plan to make the best possible use of experimental design assistants including the NC3R one (Experimental Design Assistant, NC3R) whenever appropriate.

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

We carry out pilot studies using small numbers of animals, but making sure that these group sizes can support power calculations for larger scale experiments and/or yield statistically significant results able to provide a definitive answer on whether to take forward or dismiss a particular vaccine candidate. In our work we plan to take forwards to larger animal studies only those vaccines that in pilot experiments show real promise.

Based on pilot studies and on similar work performed by us in the past, we perform statistical calculations to determine the minimal group sizes and number of repeats without compromising scientific accuracy.

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.

We shall use the mouse model of vaccination and reinfection. Animals will be immunised either with non-living bacterial vaccines or with live attenuated bacteria. The reactogenicity (side effects) of the vaccines will be extensively reduced by genetic manipulation of their DNA and is ascertained by extensive biochemical characterization and *in vitro* testing on cell lines. We have adopted the use of commercially available reporter cell lines to ascertain and measure the reduction in toxicity of the vaccines prior to inoculation into animals. We use cells lines created specifically to investigate TLR4 activation. Whenever possible, we use antibody levels and the presence of cellular responses as correlates of vaccine-induced protection (*e.g*. in the case of meningococcal and gonococcal vaccines); this abrogates the need for re-challenge with virulent bacteria. In the case of other bacterial pathogens, such as *Salmonella,* it is necessary to infect the animals with live bacteria to conclusively ascertain vaccine efficacy. In this case we only perform reinfections in those animals immunised with vaccines that have proven immunogenic in *in vitro* tests (*i.e.* measurements of antibodies and/or cellular immunity). When reinfection is required, we use low doses of live bacteria and we allow the infection to progress only for the time necessary to detect differences in bacterial numbers between vaccinated animals and non-vaccinated controls. We precisely know the grow rates of our challenge *Salmonella* strains and therefore we can be sure that the infection would not proceed faster than expected even in the event the vaccines proved to be ineffective and/or in non-immunised controls. Therefore, the challenge infections largely remain within the mild band of severity.

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

Vaccine testing requires adult animals with a mature immune system.

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

We provide the best possible husbandry care for our animals. This includes regular cleaning, health checks, within-cage enrichment tools and the use of female animals as much as possible to minimise the chances of in-cage fighting and therefore allowing group housing. We acclimatise our animals for at least 7 days before the start of any experiments. We regularly inspect the animals before and during the experimentation to ascertain that they stay in within the severity of the ascribed protocol. Experimental procedures are performed by skilled PIL (Personal Licence) holders, according to tried and tested protocols that have evolved during several decades to reduce stress and suffering to the animals. Refinements include optimisation of volumes, use individual needles for each animal, optimisation of needle size, improvement of techniques for gavage through the use of light anesthesia

that allows more accurate dosing and reproducibility of results. We reduce the potential toxicity of our candidate vaccines by removing from the bacterial DNA specific genes (e.g. lpxL1, msbB) that encode for parts of their membrane that have inflammatory activity. When we infect animals with live bacteria we always use low numbers that do not cause disease and we do not allow the experimental infections to progress to numbers of bacteria in the body that can cause, at worst, mild clinical symptoms. The numbers of bacteria to be injected are precisely measured in the inoculum by optical density and then confirmed by counting viable bacteria on bacteriological growth media. Gavage procedures performed under light anaesthesia (with rapid recovery) ensure reproducibility of experiments, reduced variability (thus need to use less animals per group) and reduced stress/harm to the animal due minimal chances of surface damage of the aesophagous (potential consequence of an animal resisting or struggling during the gavage procedure). Welfare assessment protocols will be considered when appropriate, as these can be useful tools to monitor adverse effects and determine when humane endpoints have been reached. The experience of the Named Persons and animal technicians will be useful to create welfare scores by recording relevant adverse effects and incorporating the earliest humane endpoints.

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

We follow the PREPARE (https://norecopa.no/prepare) and ARRIVE (https://arriveguidelines.org) guidelines as a routine of good practice and as a requirement for scientific publications. In addition to these, when appropriate and required we shall also benefit from papers from the Laboratory Animal Science Association, (LASA) https://www.lasa.co.uk/current_publications/. The details of each experiment (*e.g.* dosing regimens) will be based, whenever possible, on peer reviewed publications that have used similar vaccines in the past. Many of these papers are from members of our research group.

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

Our establishment provides regular updates on the 3Rs. The PPL holder is the Chairman of the Animal User's committee at his establishment and a member of the establishment Management Committee. These committee meetings include a dedicated section where improvements of the 3Rs are discussed. The PPL holder and his group attend NC3R events and workshops and subscribe to the NC3Rs e-newsletter. Regular consideration and reflection of the latest practical guidance from Laboratory Animal Science Association (LASA) will provide additional sources of new recommendations and advances in animal techniques.