



Home Office

NON-TECHNICAL SUMMARY

Vaccine development and fundamental research on viruses of medical importance

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
 - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
 - (iii) Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes
- (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, Immunology, Pandemic preparedness, Virus, Therapy

Animal types

Life stages

Mice	adult, pregnant, embryo, neonate, juvenile
Guinea pigs	adult

Animal types

Life stages

Hamsters (Syrian) (*Mesocricetus auratus*)

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?

1. To develop vaccines to viruses in animals that can cause pandemics in humans, specifically against haemorrhagic fever viruses (Ebola, Marburg, Lassa fever, CCHV) and respiratory viruses (Influenza and coronaviruses).
2. To carry out research on the above viruses in animals to discover how they interact with our immune systems. This aim supports aim 1, but will result in research that stands on its own.

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?

Vaccines are widely accepted as the best method for reducing the impact of infectious diseases, even more so than antivirals or antibiotics that treat people after they have contracted a disease. It is therefore important to future human health that we produce safe and effective vaccines to existing and especially future diseases before it's too late, and make our best efforts to exterminate them, or reduce the impact of the disease that they cause.

It is important that these vaccines work well, and work on future virus strains (future proof), ideally not requiring yearly updates or 'boosters shots' as is seen for most respiratory virus infections (influenza, COVID-19). Future proof vaccines are designed based on our prediction of how viruses will evolve over time, and to vaccinate against these predicted viruses. This also takes into account other strains of the virus that already exist, whether in humans or animals.

Basic research on viruses is also essential in animals, as viruses react differently in animals than in humans. Understanding these differences enables us to understand a lot from animal experiments and compare this to understanding virus infections in humans. This basic research can also be termed 'enabling science', that provides us with the knowledge and tools to make vaccines.

All of this work will finish in the need for studies in animals, due to the nature of viruses needing a 'host' to infect. This research is essential as to properly study a virus, one must study it in the context of a whole organism (animal) with an immune system or parts of that immune system that allow us to predict the same results in humans.

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

The primary outputs will be the generation of new, better vaccines to existing viruses, as well as broader vaccines that cover multiple viruses, or multiple strains of the same virus.

The work will produce vaccines that will be used in future grant applications that will in turn lead to more funding to expand this work. Scientific publications, patents and intellectual property will also result from this work.

Enabling science (such as supporting work on how viruses work in animal models) will generate outputs relevant to virology and viral vaccinology that are stand-alone and useful to the research community working on viruses in animals.

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

The global community will benefit from vaccine outputs, where successful. We are nearing the end of the primary part of the SARS-CoV-2 (COVID-19) pandemic, and this virus will now become seasonal, in line with other viruses such as Influenza or common cold viruses. Future pandemics are inevitable as the global population expands - our vaccines aim to reduce the risk of such pandemics happening again, by protecting people through vaccination, against viruses of pandemic potential that we have identified as circulating in animal reservoirs (similar to SARS-CoV-2). A great part of our work falls under a 'pandemic preparedness' global initiative.

Benefits from these outputs are both short and long term. Short term benefits include the development and bringing to market of vaccines within the next 5 years, such as certain of our candidates that are in phase 1 clinical trial, or other less advanced candidates that are moving towards phase 1 clinical trial.

Long term benefits include advances to the design, testing and in vivo work with vaccines that will benefit the whole field of vaccinology, and contribute to the long term improvement of vaccines over the next decades. These also include vaccines that will be brought to market after the end of this 5 year project.

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

We are extensive collaborators, and part of international consortia aimed at delivering safe and effective, broadly protective vaccines. Our grant funders are globally active charities with links worldwide and resources that will help us achieve our aims. New knowledge will be disseminated in

the form of scientific publications showcasing our work and the data generated as a result of work carried out in animals. Where possible, negative data will be published or shared with the research community, to prevent other groups from trying the same techniques and vaccines that do not work in our hands.

Species and numbers of animals expected to be used

- Mice: 7100
- Guinea pigs: 1000
- Hamsters (Syrian) (*Mesocricetus auratus*): 300

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 primarily use adult female mice, hamsters and guinea pigs to test our vaccines. Each model has its own benefits

1. adult female mice are used as the model is extremely well established, and we are experts in measuring immune responses in mice. Adult females are used to prevent fighting and to enable low stress social groups for the benefit of the animals. In some cases males will be used, and single housed where necessary to avoid fighting.
2. adult hamsters are the gold standard model for many respiratory viruses, showing similar disease to humans, and are an excellent non-lethal model of infection. Adult females are used to prevent fighting and to enable low stress social groups for the benefit of the animals. In some cases males will be used, and single housed where necessary to avoid fighting.
3. adult guinea pigs are excellent 'next step' models for testing vaccines after mice, and immune responses generated are more similar to those generated in a human. Adult females are used for the same reason as with mice, to prevent fighting and to enable low stress social groups for the benefit of the animals.

For basic virology research, we will use only mice in established or new models to measure infection, disease and innate immune parameters in these animals. For the majority of experiments adult animals are preferred, but in some cases younger animals, immune altered animals or both, in order to study viruses that establish chronic (long lasting) infections.

Animals will typically spend several months on this protocol while they build up antibody responses. This will involve several immunisation steps, and multiple bleeds to measure immune responses. Once immune responses have been measured (at which point a decision must be made as to whether these results justify continuing to the infection/challenge phase), mice will then be moved to containment facilities depending on the virus (ACL2 or ACL3) and infected, then weighed and

observed for a period of up to 14 days. Mice will then be placed under deep anaesthesia and a final blood sample taken (for final vaccine performance analysis) before they are humanely killed.

The typical experiment will involve two administrations of vaccine, usually by I.M route. Mice will be bled by superficial vein 3 times before administration of virus by I.N route. Mice will then be monitored daily and weighed, until termination of the study.

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

There are three main themes in this project license. 1. Vaccine immunogenicity evaluation (testing the immune response generated by administering a vaccine), 2. Basic Virus Research and 3. Breeding and Maintenance.

1. Vaccine immunogenicity evaluation: Testing how good our vaccines are at making immune responses in animals. Animals will be injected with 1-5 doses of vaccine (DNA, mRNA, protein or viral vector) over a study period of around 9 weeks, and serial bleeds taken at various time points (e.g. every 3 weeks) in order to measure the immune response from vaccination at those different time points. Animals may be infected with virus at the end of these studies to determine whether the vaccines protect from infection or disease.

1. For example, an animal will be given two doses of 50ul mRNA vaccine, by intramuscular (I.M) route with a 3 week interval (W0 and W3). Blood will be taken from the animals at weeks W0, W3, W6 and W9 by a superficial vessel to obtain serum that we can analyse for antibodies, and how they mature over the 9 week study.

2. Basic Virus Research: Animals will be infected with viruses in order to measure immune responses, and host-virus interactions. Animals may be given antiviral drugs or antibody cocktails to prevent or impede virus infection progression.

1. For example, an animal will be infected with a virus via intranasal route. Animals will be weighed daily and monitored twice daily until symptoms appear, usually between days 5-7 after infection. Depending on the clinical symptoms and % weight loss from pre-infection weight, mice will be humanely killed, and organs dissected - so that we can measure how the virus grew in those organs. This protocol tends to last up to 14 days after infection at which point all surviving animals are humanely killed.

At the end of experiments, animals will be humanely killed, or in the case of guinea pigs or hamsters, re homed if a suitable home is available.

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

We test a range of different vaccines and delivery methods, that themselves may cause adverse effects in animals. As vaccines are designed to generate immune responses, sometimes these can have mild or moderate effects in animals while that animal generates an immune response because of the vaccine. This is similar to how humans feel unwell when infected with a virus, for example 'flu like symptoms'.

In our experience only transient and mild discomfort is a result of immunisation with our vaccines in 99% of our animals. In some cases animals do experience mild and short lived symptoms for up to 24h after vaccine administration.

1. intramuscular injection can cause pain at the site of injection (especially in mice), which will almost always be the rear thigh.

2. Protein vaccine injection with an adjuvant can sometimes cause piloerection (raising of the hair/fur) and slight hunching for 24h after injection in mice. This is a sign that the mice are feeling unwell, but normally they get better after a few hours.

3. Infection (challenge) of naïve animals (those that haven't encountered the virus, or vaccine) with a virus can cause a range of symptoms depending on the virus. These symptoms can include discharge from the eyes, problems breathing and sickness that will cause lack of appetite - leading to weight loss in the mice. Low doses of influenza virus can cause temporary weight loss within a 10 day period, including symptoms such as hunching and piloerection. High doses of influenza virus can cause symptoms such as laboured breathing, and loss of weight leading to death. For high doses mice will be closely monitored and humanely killed after weight loss and clinical symptoms start. Any dose of SARS-CoV-2 in human ACE-2 transgenic mice will cause a rapid weight loss and moderate symptoms (such as laboured breathing, infection of the brain leading to inactivity and inappetence) within 5-7 days. There is also a chance for otherwise healthy mice to rapidly deteriorate overnight and die – due to infection of the brain by the virus, causing a stroke. In these cases mice will be humanely killed after showing moderate clinical signs or significant weight loss. In the majority of cases, mice reach humane endpoint (the point at which they should be euthanised to prevent further suffering) within 5-10 days after challenge.

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: 95%

Moderate: 5%

Severe: 0%

Guinea Pigs:

Mild: 100%

Moderate: 0%

Severe: 0%

Hamsters:

Mild: 100%

Moderate: 0%

Severe: 0%

What will happen to animals at the end of this project?

- Killed
- Rehomed

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?

Unfortunately, there is only so much that can be done in the laboratory. We employ extensive experimentation to characterise each vaccine candidate that we design, and ensure its maximum chance of success before moving into animals. Before reaching humans, a vaccine typically must be tested in multiple animal models, a very high bar that unfortunately requires quite extensive use of animals.

For basic virus research, complex in vitro (laboratory work) systems do not yet exist that match the in vivo model, and therefore once all in vitro work has been exhausted for a certain virus or project, the final steps must be carried out in animals with a view to translating results to humans. For animal viruses, the animal model may be the final or preferred model to study the virus itself.

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

There are no alternatives to animals in the field of vaccine testing, or basic virology work. Ultimately these need to be tested in an animal that has a working system of organs, immune system, microbiota (normal bacteria, fungi and viruses that make up part of our body systems). As viruses are obligate intracellular pathogens (they can only survive inside our cells), they require a host, and while cell based systems are useful for very basic research, the whole organism is where the majority of scientific findings are discovered. This is especially true of viruses that infect animals with incredibly complex immune systems such as mammals.

We work extensively with a range of cell culture lines in the laboratory, as well as using biochemical and biophysics experiments to evaluate our vaccine candidates. These enable us to whittle down our vast libraries of candidate vaccines until a select few candidates that fit the expected or desired immune profile are used.

For basic virus research, only cell culture based systems can be used, as viruses can only function inside living cells. We employ these in our day to day experiments working on these viruses. These

are employed to grow and measure virus, and in some cases to image parts of the cell that the virus interacts with.

Why were they not suitable?

Cell based systems are suitable for a range of experiments, but are very different to in vivo work, where a whole animal has various cell types, organs and a working immune system. It is unfortunate that cell based systems are currently not at an advanced stage to replace animal work, and this will remain the case for the next century or longer.

They are not suitable as they provide a small amount of information, but not information on the immunogenicity or characteristics of a vaccine or viral infection in a whole body organism.

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?

In the past two years, we have successfully executed three major vaccine projects involving large amounts of animal work.

- Influenza universal vaccine project (£4m): Total of ~1500 mice
- Coronavirus vaccine projects (£2m): Total of ~1500 mice and 130 Guinea pigs
- Haemorrhagic fever virus vaccine projects (£500k): 28 Guinea pigs and 100s of mice

Based on these figures for two years, and taking into account that we have just been awarded £44m to design a pan coronavirus vaccine, the projected animal numbers for a 5 year project, continuing at our current rate of animal work, would be ~7200 mice and ~300 Guinea pigs.

We have decided on 7100 mice, 1000 guinea pigs and 300 hamsters due to our pan coronavirus vaccine project requiring more Guinea pig work. This factors in a slightly lower number of mice than predicted, but also includes mice for breeding and maintenance protocols. Hamster numbers are for exploratory studies and can remain low at 300. This also takes into account non-vaccine work to develop animal models for newly discovered beta-coronaviruses that pose pandemic threats. This typically requires much fewer animals per project.

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

1. The vast majority of our studies are pilot studies. We do not know how the vaccines will perform, and the reagents to test how they have performed are not regulated or well defined in the research community. Therefore we cannot carry out power calculations that need a defined effect and set of hypotheses and we must use other means to reduce our group sizes.
2. All vaccine candidates are tested in vitro, and quality controlled (tested in the laboratory to make sure they are correct), enabling us to select the best candidates to take forwards to in vivo work. As there are very many variables in vaccine studies, it is only possible to perform power calculations in retrospect, when building on a previous study. Therefore it is not possible to do this for pilot studies where the variance and interplay between groups, controls in experimental assays - is unknown. The majority of our vaccine studies fit into this group of pilot studies. We use the below formula to ensure that we do not use too few animals per group and per study:

$$E = (\text{number of animals per group} \times \text{number of groups}) - \text{number of groups}$$

An E number of between 10 and 25 is within the correct range for statistical analysis, below 10 being too few animals and above 25 being too many animals. We will always aim to be on the lower side where possible.

1. Power calculations are carried out where possible to guide animal numbers for vaccine and basic virology research experiments. Where possible, animal numbers are minimised in this respect.
2. All efforts are made to make each animal study fully controlled and publishable in its own right, with the minimum number of control animals required to allow us to have confidence in our results.

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

As this project license builds on a previous similar license, we are able to utilise our expertise in the field of in vivo vaccine and virus work to optimise the number of animals used in our projects. Our studies flow through an exhaustively detailed and planned pipeline from the design stage until endpoint in humans.

The majority of our vaccine studies can be considered "pilot studies" as we are measuring immune responses to new vaccines. They are computationally designed and quality checked, then go through several rounds of in vitro quality control. After this, the refined list of candidates is much reduced and can progress into animals.

When infecting mice with a new virus, or immune altered mice with an existing virus, there are very many unknown factors, which forces us to carry out pilot studies with the fewest animals possible in order to build up knowledge about the viruses and how they work in animals. This builds on our existing knowledge, and helps us refine future studies with similar viruses or vaccines.

Through our use of standardised protocols developed over the past 5 years, we are able to reduce bias and account for variation when vaccinating animals. We have tested these standardised protocols and shown that results are reproducible over multiple studies. This gives us certainty that the small numbers of animals used in our groups is sufficient.

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 use a very simple immunogenicity protocol for the majority of our animal work, involving 1-5 injections of vaccine, and up to 8 bleeds at various time points in the vaccination schedule. These are mild protocols that cause very limited pain, suffering or distress - and no lasting harm to the animals. Our guinea pigs can and have been rehomed after being on such protocols with permission from the HO.

For basic virus research, we use wild type, and sometimes modified mice to interrogate different aspects of virus-host interactions. The most we can do in this case is administer a low dose of virus and be especially vigilant to humanely kill mice well before any humane limit is reached. This is an unpredictable aspect of virology, in the few cases when mice react badly to infection or animal checks fail to identify sick animals, they may breach the severity limit of mild and we will be required to submit a SC18 report. We never have a scientific need to keep an animal alive after breaching the severity limit and this will be avoided at all costs.

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

We require animals with mature mammalian immune systems that are capable of mounting an immune response to an infection or vaccination that can take months to manifest. Only mammals are suitable as they are models for vaccines in humans. There exists no cell-based model or less sentient model to test vaccines.

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

Our establishment and associated animal houses provide state of the art, and the most modern housing for our animals, with an emphasis on their wellbeing.

While females are used in most cases to allow larger social groups and prevent fighting, males are housed separately where needed and in the case of fighting. Bullies are also separated on welfare grounds. Animals are allowed 7 days to acclimatise before procedures, and our best efforts are made to reduce the number of procedures carried out in total, or to restrict these procedures to a minimum number of time points (or stress points) to reduce long term stress. Increased monitoring is carried out on moderate protocols, or those including virus infections, with animals monitored at least twice daily, weighed and a clinical scoring sheet used to aid assessment of the animal's health. In human ACE-2 transgenic mice, any clinical signs that deviate from normal will lead to animals being humanely killed.

For other mice where sickness is more predictable, animals will be allowed the opportunity to recover from infection, and only humanely killed when it is clear that symptoms are not going to resolve in short period of time.

The procedures we use are always re-evaluated based on the expertise available, we have recently moved to using dark plastic restraining tubes and seen a marked decrease in stress in mice being sampled. We have also moved to more streamlined protocols, meaning that the majority of our study plans will include only three bleeds and two immunisations, rather than 5-7 bleeds and 4 immunisations. However, some studies may require the full set of bleeds and immunisations depending on the research outputs and vaccines used.

As GM strains of desired mice become available from commercial suppliers, we will opt to order cohorts in directly rather than breed these mice ourselves - this should refine our ability to produce data at the least cost possible for the animals, as companies are better placed to breed in an efficient manner and make full use of the resulting animals.

For monitoring purposes, we have refined how we deal with mice that are expected to develop clinical symptoms (e.g control unvaccinated, infected animals), in liaison with animal staff at our facilities. In such cases, a researcher will be ready to humanely kill animals on all of the expected symptom days, rather than waiting to be called in by animal staff after a monitoring session has raised concerns about the symptoms shown by an animal. This refinement reduces the amount of time spent suffering by an animal to a minimum. In addition, we will perform data analysis in the laboratory before infecting animals to test whether vaccines work or not. If our data suggests that the vaccines have not worked, or that the challenge does not fulfil the scientific objectives of the licence, then the infection/challenge step will not be performed.

Where possible, guinea pigs and hamsters will be rehomed where a suitable home is available.

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

All of our animal work is carried out within a framework provided by our establishment, ensuring that we have the most up to date information to plan our studies, and that our experiments are planned with the latest guidance in mind. We will keep up to date on the latest LASA publications, NC3Rs 'ARRIVE' guidelines, NORECOPA 'PREPARE' guidelines. We will also keep up to date with latest RSPCA studies (e.g. <https://science.rspca.org.uk/-/refining-procedures>) to refine our protocols and animal use.

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. We also receive regular updates on the 3Rs through the NC3Rs website/NC3Rs Gateway (<https://f1000research.com/nc3rs>).

We regularly monitor the scientific literature for advances or breakthroughs in the animal work relevant to virology or viral vaccinology, in particular paying attention to the development of in vitro models that may allow us to reduce or refine our animal work. We will also review journals such as the ATLA

(Alternatives to Laboratory Animals) Journal: <https://journals.sagepub.com/home/atla>, and refer to the LASA Guidelines: https://www.lasa.co.uk/current_publications/