Home Office

NON-TECHNICAL SUMMARY

The development of in vivo expressed biologics as a platform for novel therapeutic agents

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

DNA, RNA, Delivery, Molecular switches, AAV

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?

We are interested in developing new technologies that can enable and assist delivery of therapeutics (medicines that heal disease), encoded in DNA or RNA molecules either alone or within viral or non-viral particles, with precise control over when and where it is expressed (turned on) in patients. This work aims to increase our understanding of the duration of expression and activity of the delivered transgene (artificial gene), cell or organ targeting, host responses, and how to precisely control therapeutic activity using molecular switches.

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?

Due to population growth and increased longevity, the total number of deaths attributable to non-communicable diseases (NCDs); diseases that are not caused by an infectious agent, has risen over the last 20 years. Cancer, cardiovascular disease, diabetes and chronic respiratory diseases killed approximately 33.2 million people worldwide in 2019, a 28% increase compared to 2000. NCDs continued to be leading causes of ill health worldwide and were responsible for seven of 10 premature deaths in 2019 (from WHO World health statistics report 2022). Therefore there is a clear need to pioneer new medical treatments and technologies to address this increasing disease burden. This work will aim to develop several innovative drug delivery platforms by overcoming the existing technological hurdles or otherwise improving the expression, targeting, immunogenicity (the degree to which the immune system recognises it) or control of the expressed DNA or RNA molecules. This could enable us to produce new medicines that are more effective, longer lasting and with fewer side effects than is currently available.

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

The primary output of this work will be a greater scientific understanding of how to achieve peak performance with new therapeutic delivery technologies in vivo (in a living organism). This could include expression parameters (how much and how long the therapy is present in the organism, or pharmacokinetics), how to target these molecules to a particular cell or organ, how to dampen immune responses, or how best to control the activity of these molecules in vivo. Many of these technologies will be at the cutting edge of the field, and thus the work will determine the future viability of these molecules in vivo. This will inform specific drug projects that are aiming to produce new medicines for patients. Where possible this work will be published in scientific journals, presented at conferences, or used in the creation of patents.

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

In the short term the work will show us which parameters, vector (the vessel that carries the gene) designs or molecular sequences create the best possible performance in vivo for novel therapeutic drug delivery platforms. In the medium term this will allow us to choose the best designs or methods to take into full pre-clinical (before testing in people) and clinical (testing in people) drug development in order to create novel or improved medicines. In the long term this will benefit patients in multiple therapy areas including cancer, lung disease, heart disease, brain disorders, or infectious disease, and many others. Furthermore the papers published from this work will enlighten and benefit the entire scientific and medical community working on similar technologies.

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

This work is a collaboration of many internal teams, working on different aspects of these technologies, and we frequently meet to discuss results, and how to link our discoveries and learnings across the teams. We also have collaborations with academic labs and technology providers, broadening our scope further. Where possible we will publish the knowledge gained to the wider scientific or medical community, as papers or as presentations at conferences.

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.

Mice are the preferred species for our studies because they are small and easily-handled mammals with a highly characterised and well-defined biology. This makes them an excellent model for this work, as they will model the expression, immunogenicity and targeting of our technologies very well. They can also be genetically altered in order to better study or reproduce human disease, or allow us to assess our technology in a more human-like context. For instance, there are several mouse strains that are modified to not mount an immune response to human cells, which allows us to implant human tumour cells or even human cell therapies without fear of these being rejected by the mouse host. We don't require juvenile or aged mice, as mice of adult age are perfectly adequate for this work.

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

Animals will be bought from suppliers and transported to the facility at around 8 to 12 weeks of age. They will then be moved into cages in groups of 3-5 animals, with constant access to food and water, and lots of enrichment including multiple types of nesting and/or bedding material, plastic tunnels, pyramid houses, and wooden chew sticks. Both male and female mice will be used for this work, with

the sexes kept apart in their groups of 3-5 animals. We expect female mice to remain in these group sizes without incident, but at times males may need to be housed separately to avoid fighting. Once on study animals will typically experience mild, transient pain and no lasting harm from administration of substances by injection using standard routes, for example via intravenous (into a vein) or intramuscular (into muscle) injection, with electroporation (the application of a short pulse of electric current) for the latter. When mice are given intravenous injections they will be restrained fully awake in a clear plastic tube, and typically mice will receive only one intravenous injection in their lifetime. For intramuscular injections the mice will be briefly anaesthetised (put to sleep) during the procedure (for around 5-10 minutes), and will typically receive only one intramuscular injection in their lifetime. Most other injections will be given while the animal is restrained by 'scruffing', such as subcutaneous (under the skin) or intraperitoneal (within the peritoneal cavity) injections or oral dosing. When these administration methods are used animals will typically receive 5-10 total doses in their lifetime from one of these routes only. Occasionally animals will be implanted with tumour cells via a single subcutaneous (under the skin) injection while under anaesthesia (duration of around 5-10 minutes), and this will be accompanied by microchipping for identification (also applied subcutaneously). After the first procedure mice will typically remain on study for approximately 3 to 6 weeks. During this period, serial (one after the other) blood samples may be taken via the tail vein (typically 2-3 per animal), or the mice may occasionally be anaesthetised for around 20 minutes for the purposes of imaging (typically 1-2 times in a lifetime). In nearly all cases animals will either have blood samples taken or be imaged, and not both, as generally only one of these methods will be appropriate to monitor the expression of the test article in each case. Rarely, mice will undergo changes in diet which are not expected to cause distress, but may result in weight loss due to unpalatability. At the end of the study mice will be humanely killed, and tissues may be taken for ex vivo (outside of the living body) analysis.

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

Animals are expected to experience only mild, transient pain and no lasting harm from administration of substances in most cases. The use of anaesthesia when dosing the animals will be used when appropriate to further reduce the pain the animal will feel. Occasionally animals may exhibit signs of ill health following administration of substances, such as piloerection (bristling of fur), inactivity, or hunching. Mice that show one or more of these signs will be given supportive treatment such as warming or supplying extra bedding or enrichment, and if signs do not resolve the animals will be culled before the limits are exceeded. Unexpected weight loss, another sign of ill health, may also be observed occasionally. Weight loss will be measured against the highest weight recorded for each animal, and in most cases mice will be given diet supplements to help the weight loss recover before limits are reached. As the substances that will be tested on this project are designed to be as harmless as possible for the recipient, the majority of animals (60%) will be studied on a mild protocol, which has a low limit on the duration of signs of ill health (less than 24 hours) and weight loss (10%). The remaining animals (40%) will be studied on a moderate protocol, which has a slightly higher limit on the duration of signs of ill health (less than 48 hours) and weight loss (15%). Very occasionally mice on the moderate protocols will be allowed to exceed 15% of body weight loss, providing there are no other clinical signs, and the weight recovers to less than 15% within 48 hours following the substance administration. This will only be experienced by a minority of mice on the moderate protocols (less than 20%). In the case of tumour studies there will be additional limits on tumour volume and tumour condition that will ensure animals are not experiencing discomfort or signs of ill health for a period greater than 48 hours.

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: 60% mild, 40% moderate.

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?

While a range of in vitro (in a test tube, culture dish, or elsewhere outside a living organism) assays will be performed by our research team to triage (decide the order of) molecules or vectors prior to testing in vivo, animal models are needed because in vitro or in silico (modelled in a computer) systems cannot fully capture the complex spatial-temporal (over time and space) pathways that will determine the pharmacokinetics (amount of the compound present in the body over time), pharmacodynamics (the effect of the compound on cells in the body) and biodistribution (where in the body the compound goes) of these novel therapeutic technologies or substances.

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

We considered the use of human and/or mouse cells in culture either as single layers grown on a dish, or as organoids (3D cell culture) suspended in media. We also considered the use of computer modelling (i.e. in silico) technologies.

Why were they not suitable?

There are fundamental limitations to the in vitro culture of cells or organoids for this work. For instance there is no way to model the biodistribution at a systemic level (i.e. which organ does the substance go to), nor the complete host immune response, as those complex systems are not currently possible to adequately reproduce in culture. Similarly, while in silico technologies are continuously improving, it is yet to get close to capturing the full complexity of a biological organism like a mouse or human.

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?

This estimate is based on previous in vivo work performed by the relevant teams on other licences in the past, combined with a projection of the number of studies that are planned to be run over the next five years, and the approximate mouse numbers required in each study. Approximate numbers are based on recommendations from in-house statisticians on what would be appropriate powering for the studies I have run over the past 18 months on other institutional licences, or studies that are planned in the future.

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

The most appropriate models and experimental design were selected only following a review of all in vitro and in silico analysis and the scientific literature. Furthermore, consulting with statisticians in the study design phase is a requirement for compliance with Company Global Policy on Good Statistical Practice (GSP). For example we will always use the minimum number of animals required to achieve a significant biological effect as determined by a power analysis for every study. Study designs will also include positive and negative controls, where relevant. This statistical approach ensures sufficient data is obtained from each study, negating wasteful repeats, but also preventing superfluous treatment groups or excessive group sizes. Randomisation will be incorporated into studies, based on either body weight or tumour size, and blinding (an experimental design where the user is unaware whether an experimental group was given a treatment or a control substance to avoid user bias) will be performed where possible, especially on samples collected for ex vivo analysis.

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

This work will make extensive use of pilot studies, as most of the test substances will be by definition novel, as they concern novel technologies or modalities (method of treatment). Thus pilots will be run routinely to discriminate new vector designs or sequence modifications with small group sizes intended to give an initial steer. Only when results from those pilots are positive will larger studies be designed in order to refine the data that can be obtained. For pharmacokinetic and some pharmacodynamic studies, animal numbers will be minimised through the use of serial sampling wherever possible. Biodistribution or tumour studies will make use of in-life imaging of fluorescent (glows a bright colour under certain light) or bioluminescent (emits a bright light like a glow-worm or firefly) reporters when appropriate, allowing several data points to be obtained from a single animal throughout the experiment. To further reduce animal use, when endpoint is reached we will take and appropriately store numerous tissues in order to perform extensive ex vivo analysis. This will include splitting tissues into several parts in order to fulfil multiple assays, such as fixing for IHC (immunohistochemistry) analysis, or freezing for DNA/RNA extraction.

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 are the preferred species for our studies because they are small and easily-handled mammals with a highly characterised and well-defined biology. The majority of studies will use wild-type (not genetically modified) mice, but the genetically altered mice we do use will be immunodeficient (lack parts of the immune system) strains such as SCID (Severe Combined Immunodeficiency) mice that show no significant physiological abnormalities and do not exceed mild severity. Most studies will not induce disease, except for the minority of studies that involve the establishment of subcutaneous tumours. For the typical study the number of procedures will be minimal with a single injection of the test article, followed by up to 6 tail bleeds or imaging under anaesthetic. This serial bleeding or imaging to measure pharmacokinetic (PK) or biodistribution respectively will reduce the numbers of animals required per study. Where tumour cells are implanted into the mice this procedure will be performed under anaesthetic to minimise pain or suffering. Pilot or tolerability studies will be carried out for new models or test agents respectively to minimise the number of animals impacted by any unexpected adverse effects.

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

The mouse is among the least sentient mammalian species that is available for routine in vivo work. Non mammalian species, i.e. flies or zebrafish, would be too distant from the human for the results to be usefully translated into patient use. For example, similar tissue types and tissue structure, a similar circulatory (blood) system, and a similar immune system, are required for the results of the in vivo work to be translatable into the human context. This work cannot generally be performed on terminally anaesthetised animals, as the studies need to last for several days or weeks for us to study the pharmacokinetic (PK), pharmacodynamic (PD) or biodistribution of these test articles over time.

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

When planning experiments I will consult relevant published guidelines, including the PREPARE guidelines checklist. I will also check the NC3R online resource library for the most up to date information on refined procedures, including dosing or sampling techniques. After transportation from the supplier to the facility, animals are given an acclimatisation period of at least one week prior to being placed on study. Tumours are measured with callipers but also monitored for changes in appearance, such as redness or scabbing, to flag potential issues before they arise. Tumour-bearing mice will be assessed using the body condition scale where body weight monitoring may not be sufficient. All mice will be assessed according to the grimace scale where appropriate. Animals are

group housed in cages of 2-5 mice depending on weight and sex, and we avoid lone housing unless absolutely unavoidable (i.e. fighting in the group). Animals will be given lots of enrichment as standard, and extra bedding can be provided following procedures. Our technicians are all experienced users of animals and use non-aversive handling techniques. When adverse effects or weight loss is recorded in any of the studies, the observation of the mice will increase to monitor any progression of the pain and suffering of the animals. Early interventions may be given where necessary to minimise the potential for progressive weight loss, such as giving a mashed diet before or after article administration. This will ensure acceptable severity parameters are not exceeded. Post-mortem examinations will be performed to investigate unexpected deaths. Where relevant, tumours will be studied at the earliest stage of growth compatible with a meaningful result to the experiment.

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

Unless otherwise justified studies will be carried out to best practice as described in Workman et al (2010). Other published best practice guidance that is familiar within the establishment are the Handbook of Laboratory Animal Management and Welfare (Wolfenson & Lloyd) and LASA best practice guidelines. I will also refer to the PREPARE and ARRIVE guidelines.

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

I will stay in touch with my local in vivo network of companies, universities and animal facilities, that have regular meetings. I will regularly check information on the NC3Rs website, sign up to the NC3Rs newsletter, and will attend regional 3Rs symposia. I will also establish regular contact with my named persons in the animal establishment (NVS, NACWO and NIO). Advances in the 3Rs will be incorporated into my studies at the design stage by informing the scientists designing the studies on this project, and at the implementation stage by informing the technicians carrying out the hands-on in vivo work. This will be communicated at regular team meetings within the in vivo team.