Home Office

NON-TECHNICAL SUMMARY

Investigation into the therapeutic potential of exosomes

Project duration

5 years 0 months

Project purpose

- (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

Exosomes, Therapy, Drug Targeting, Bioengineering, Rare disease

Animal types	Life stages
Mice	adult, juvenile
Rats	adult, juvenile

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?

To test the therapeutic potential of a type of naturally occurring molecule secreted by human cells (exosomes). These molecules will be investigated as a novel way to deliver targeted therapies for treatment of human disease.

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?

The majority of existing drugs are distributed throughout the body based on their physical and chemical properties. This approach is usually associated with drug reaching its therapeutic site of action but not always at sufficient concentrations to have a therapeutic effect. Increasing the dose may result in toxicities that limit the amount that can be given. Newer approaches with biological agents including antibodies, cell and gene therapies offer potential to get around this challenge as they are more specific to their target within the body. However, these agents suffer from different challenges, including adverse immunological responses, issues with repeat dosing and challenges around getting the drug to the part of the body where the target is found. We are investigating the potential of exosomes - a type of molecule secreted by the majority of cell types in the human body. In the body these molecules play an important role facilitating different cells communicating with each other. The molecules are found in blood and are administered to humans as a natural component of blood during blood transfusions. They can also be derived from cells in the lab and modified to contain drugs either by encapsulating them or carrying them on their surface (or both). The approach we are taking to produce exosomes in the lab will utilise human cell lines. Because these molecules are derived from human cells, they do not suffer from many of the unwanted properties of other biological drugs, such as eliciting immune responses. To determine how best to apply these molecules to treat human disease, we need to further characterise their properties including where they go to in the body following different dosing routes, whether they can deliver sufficient amounts of drug to the target cells and how long they last.

Once we achieve this, the initial therapeutic area of interest is rare diseases such as argininosuccinic aciduria (ASA), Pompe disease and phenylketonuria (PKU). These diseases either have no treatment or poor treatment options, are often detected very early in childhood life and severely affect lifespan and quality of life.

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

- data to understand the unique properties of different modifications of exosomes and how to apply these to the biggest areas of unmet therapeutic need

- data for informing design of preclinical regulatory testing as a precursor to first in man / clinical trial and potential novel therapeutics

- publications to disseminate new findings relating to the use of exosomes as a therapeutic platform

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

Short term the company will benefit by obtaining data to best position our molecules and enable preclinical regulatory studies and clinical trials.

Medium to longer term it is anticipated that there will be treatment or correction of rare diseases either for which either there are no therapies or where there is significant benefit compared to the current standard of care.

Longer term it is anticipated that treatments will be developed for a broader range of human diseases.

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

Study design will incorporate maximum use of tissues and body fluids (e.g. storage of samples for later studies) from each animal.

Cadaver material not required for the current project may be used by the facility for other researchers / teaching under the establishments tissue sharing policy.

Species and numbers of animals expected to be used

- Mice: 1000
- Rats: 200

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 will be used for most experiments because they are the smallest animals with the appropriate physiology for studying biodistribution. Due to their size they also require less test article. Furthermore, if test articles progress to disease models, the majority of these are genetic models in mice and so this avoids repeating experiments in different species. Rats may be used for studies where the small size of mice makes administration of the test article technically difficult; most likely studies requiring direct dosing into the CNS. Adult animals will be used to enable collection of larger volumes of blood and more tissue for ex vivo analysis, thereby reducing the number of animals needed.

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

Animals will be administered test article (or control material) either by a single or multiple injections and monitored over time, including blood sampling. Blood will also be collected under terminal anaesthesia and tissues harvested after death. Blood sampling will be based on the guideline volumes provided in the NC3Rs decision tree:

https://nc3rs.org.uk/mouse-decision-tree-blood-sampling

https://nc3rs.org.uk/rat-decision-tree-blood-sampling

Typically an individual mouse will not provide more than one in-life blood sample. Rats will typically provide multiple blood samples via a temporary canula.

Where multiple injections are required, the frequency of dosing will depend on the duration of effect observed following administration of a single dose. It is not expected that this would be more regularly than once every 3 days for 30 days or once a week for 60 days.

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

Previous data from our company and other researchers (example publications below), suggests that the test articles should have a clean safety profile and therefore adverse effects are limited to pain caused by administering drug or from blood sampling.

Escudier B., Dorval T., Chaput N., Andre F., Caby M.P., Novault S., Flament C., Leboulaire C., Borg C., Amigorena S., et al. Vaccination of metastatic melanoma patients with autologous dendritic cell (DC) derived-exosomes: Results of thefirst phase I clinical trial. J. Transl. Med. 2005;3:10.

Morse M.A., Garst J., Osada T., Khan S., Hobeika A., Clay T.M., Valente N., Shreeniwas R., Sutton M.A., Delcayre A., et al. A phase I study of dexosome immunotherapy in patients with advanced non-small cell lung cancer. J. Transl. Med. 2005;3:9.

Besse B., Charrier M., Lapierre V., Dansin E., Lantz O., Planchard D., Le Chevalier T., Livartoski A., Barlesi F., Laplanche A., et al. Dendritic cell-derived exosomes as maintenance immunotherapy after first line chemotherapy in NSCLC. Oncoimmunology. 2016;5.

Suzuki E., Fujita D., Takahashi M., Oba S., Nishimatsu H. Stem cell-derived exosomes as a therapeutic tool for cardiovascular disease. World J. Stem Cells. 2016;8:297–305.

Dai S., Wei D., Wu Z., Zhou X., Wei X., Huang H., Li G. Phase I clinical trial of autologous ascites-derived exosomes combined with GM-CSF for colorectal cancer. Mol. Ther. 2008;16:782–790.

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)?

100% mild severity

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 understand the properties of the molecules in the whole body, including how long they remain in the blood, where they go within the body and how long they stay in different tissues.

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

There are no non-animal models currently available that would address the same questions and provide the necessary data needed for first time in human studies. As we generate data we will explore any in silico methods that become available e.g. for predicting biodistribution. In vitro models including organ on a chip models will continue to be reviewed to determine if these can replace animal use in the future e.g we are currently investigating in vitro models to assess blood brain barrier penetration. We also have a collaborative project to test pig and human livers that are by products from the meat industry or unsuitable for organ transplant, respectively.

Why were they not suitable?

Currently these models are not validated to the extent that they are ready to replace whole body experiments.

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 numbers have been calculated based on the preliminary information on group sizes to give statistical power obtained from contract research organisations. This has then been multiplied by the anticipated number of groups to cover the routes of administration to be tested, targeting strategies, time points and lead candidate therapeutics to be tested.

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

Testing as many parameters as possible in vitro to minimise animal testing.

Comparing multiple groups in a single study to avoid duplication (particularly of control animals).

Aiming to miniaturise assays to use less sample and therefore make more measurements from the same animal e.g. for plasma.

We will use the NC3Rs Experimental Design Assistant and statistician support (Ref: https://www.nc3rs.org.uk/experimental-design-assistant-eda). As well as additional statistician support.

Power calculations based on data from externally run studies and collaborators will be used wherever possible.

Reverse translation from clinical studies to ensure minimal in vivo studies to support clinical testing.

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

Use of data from previous and current studies being run at contract research organisations and with collaborators.

Analysing data from single dose studies before planning repeat dose studies.

Tissue and data will be shared across projects within our group or offered to other groups.

Time course studies maximising in life end points to reduce the number of terminal measurements requiring groups at each time point.

Minimising sample volumes needed for analysis (miniaturisation or highly sensitive assay methods) to reduce replication of animals due to restrictions in sampling volumes.

Evaluate newer methodologies as they arise, particularly around in silico predictions.

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 animals used will all be healthy animals and will only undergo procedures to administer therapeutic or control molecules or to sample blood before terminal procedures. Studies will be designed to minimise the volumes of blood needed and will use temporary cannulas where repeat samples are needed with short intervals. Use of a new needle for each animal will be carried out on

every study. When testing effective routes of administration, the route with the least pain, suffering and distress will subsequently be used where different routes provide comparable data. The volumes of material injected will be the minimum volume necessary and infusion methods will be considered if larger volumes are needed.

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

The animal models selected are the least sentient available for the project.

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

General anaesthesia will be used for routes of administration associated with greater pain and where multiple blood samples are needed in a relatively short space of time.

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

We will follow the NC3Rs guidelines on the "Responsibility in the use of animals in bioscience research" and consult any relevant references listed therein. (Reference: NC3Rs/BBSRC/Defra/MRC/NERC/Royal Society/Wellcome Trust (2019) Responsibility in the use of animals in bioscience research: expectations of the major research councils and charitable funding bodies. London: NC3Rs.)

We will follow the Good Practise Guidelines as set out by LASA (Reference: https://researchanimaltraining.com/wp-content/uploads/2021/05/lasa_administration.pdf).

Animals will be monitored for signs of pain and distress as required by experienced veterinarians and animal care technicians with significant experience in these species.

Standard Operating Procedures are employed for animal husbandry and procedures.

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

We will continuously monitor publications and the NC3Rs website for new and alternative models that could be implemented as part of this project. In addition, articles on advances in the 3Rs are regularly published on RVC News Forums and other relevant information is circulated by AWERB as relevant to PPL Holders. Whenever possible we will implement these refinements into our studies.