Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

Our procedures has been well stablished within the scientific community and are only considered of moderate harm for the animals. Therefore, our protocols are only considered of moderate harm to the animals. However, adverse effects could appear. In some cases we will need to anaesthetise the mice to perform protocols to deliver gene and cell therapies to eyes of brain. They could be infections, haemorrhage or unexpected secondary effects of injections and anaesthesia. On top of the injection procedures, there is a lot of information and plenty of data suggesting that regarding the vectors and cells we inject have no side effects and, proving they are very safe. We will design the experiments in advanced and we will know all the different tests we will want to check in treated animals after our treatment. After the test, we will kill the animals to further do more analysis and validate our approach to treat the disease. The final expected level of severity will be always moderate. We aim to be vigilant if unexpected severe adverse signs appear to euthanize the animals.

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 species)?

This project only will use mouse as model animal. In this project half of the animals are expected to have a mild severity and the other half of the animals a moderate severity procedures.

What will happen to the animals at the end of the study?

- Killed
- Used in other projects

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?

Ciliopathies are inherited genetic diseases and are mostly multi-system disorders, and disease of one tissue or organ often has knock-on effects on disease in other organs or tissues. For example, in Bardet-Biedl Syndrome present brain pathology and in in the hypothalamus thatcauses massively enlarged body weight and hormonal misregulation.

We have demonstrated that correction of brain function has a direct impact in fat accumulation. Therefore, to identify the correct target for treatment we require a complete organism, a living animal,

and is something that cannot be achieved solely by in vitro or cell culture analysis.

Moreover, if you need to proof efficacy of your gene replacement product the only method accepted by all drug regulatory agencies (UK - Medicines and Healthcare products Regulatory Agency (MHRA), USA - Federal Drug Administration (FDA), EU - European Medicines Agency) is to demonstrate it in an in vivo whole animal model.

What was your strategy for searching for non-animal alternatives?

Nevertheless, all constructs are initially tested in cell culture using human cells lines. This is firstly to assess that the vector is functional and capable of expressing the transgenes in the manner it was designed for. When using vectors able to transfect in vitro cell lines (e.g. lentivirus), batch quality control analysis are performed to ensure that a gene therapy preparation is of a sufficiently high titre. These in vitro alternatives will always be used before any in vivo analysis.

Moreover, we are also continuing to investigate how human IPSC-derived cell cultures can be used to study gene therapy effects on different human cell types in vitro. Those studies have already developed three-dimensional organoids and esferoids such as eyecups and brain organoids that recapitulate some cell layers and structure of the tissues. However, those still cannot replace the metabolic interactions between different organs found in animals.

Why were they not suitable?

The alternatives described above will allow us to test the improved methods and materials in vitro. This way we will know which are the most promising constructs, cells or combinations of methods allowing us before we start the studies in our animal models.

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 m lpteċurrprojeerþieσ Disn

We have estimated the numbers of animals used based in our previous experience with this type of mouse lines. We also know the breeding requirements of our mouse lines. Our approach to the study and development in our research programmes allow us to calculate with precise accuracy what are the number of studies and the number of animals. For each programme we have tight timelines that ensure we won't over or underestimate the resources we need to compete them.

What steps will you take to reduce animal numbers? Wh]o Ô]ngeWhas]r or unde]nde]btake to

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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.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

Mice will be the animals used in this project. To investigate human genetic conditions mice are the best and only model. Mice are mammals, they share most of the genes with humans, when we mutate a gene in mice it usually shows the same symptoms as the patients. We even have models that have the exact same mutation changed that is found in some of our patients, and they have the same defects we find in humans.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

We will follow the updates on the ARRIVE guidelines published (https://arriveguidelines.org/). For surgery we will follow the LASA Guideline or Preparing for and Undertaking Aseptic Surgery (https://www.lasa.co.uk/wp-content/uploads/2017/04/Aseptic-surgery-final.pdf)and follow their news, updates and recommendations (https://www.lasa.co.uk/info/). We will also refer to the PREPARE guidelines: (http://journals.sagepub.com/doi/full/10.1177/0023677217724823]) and its updates.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

Our lab is in close contact with the N3CRs, submitting grant applications to improve the therapeutic methods, with submissions to CRACK IT Challenges (https://nc3rs.org.uk/crack-it-challenges).