[redacted]

NON-TECHNICAL SUMMARY

Breeding and therapy of the DE50-MD dog

clinical trial. In contrast, dystrophic dogs show similar clinical and pathological progression to humans

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.

There are various animal models of Duchenne Muscular Dystrophy that have advantages and disadvantages for research. Mouse models in particular are useful for initial screening of drugs, but they generally do not display clinical signs of disease so they are not suitable for testing treatments designed to promote functional improvements. Pig models of DMD (kept in other countries) have a very severe phenotype and most die or are euthanased within the first few weeks of life. They are therefore unsuitable for testing treatments in longer duration trials which are important for prolonged efficacy and safety evaluation.

We will use dogs with a naturally-occurring form of Duchenne Muscular Dystrophy caused by a mutation which is in the identical gene that is mutated in humans with the same disease and in a region of the gene that is most often affected in humans. We will only breed animals that are required for maintaining the colony, studying the disease and for the therapeutic trials. We will study animals throughout their lives - up to approximately 1-2 years of age. The colony will be maintained for the duration of the programme. Additional healthy animals that are bred that are not required for the trials will be rehomed whenever possible, usually after weaning as is done for pet dogs. We have placed over 120 dogs in this way in our prior work since 2016.

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

Generally the procedures conducted on dogs within this project are no dif

medication as recommended by vets. Other procedures are not expected to be associated with any discomfort. Some procedures (for example MRI imaging) are conducted under general anaesthesia.

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

Therapies might have first been tested in cell culture before animal studies or might have been tested by other groups using organoid type preparations.

Why were they not suitable?

Our work covers the final investigations that are required before moving into therapeutic trials in humans. Cell culture studies are often used in early investigations but they are not suitable for assessing final therapy development as it is currently not possible to generate mature muscle and other relevant tissues in a cell culture system because cultured muscle cells fail to differentiate to become mature muscle fibres that are found in live mammals. Cultured cells for example might lack the relevant receptors necessary for drug entry into a muscle fibre. Even organoid-type preparations do not have a mature blood or nerve supply or the immune system and local tissue fibrosis that has to be taken into account when doing our work.

A retrospective assessment of replacement will be due by 28 March 2028

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

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?

For each study we will use the minimum number of animals that are required to prove or disprove the efficacy of a possible treatment. We have already performed very extensive testing that allows us to know with high confidence the minimum number of animals that are required to produce a robust result. For example, we might be interested in determining whether a specific treatment can improve an affected animal between 25% to 50% towards normal for a specific issue (such as muscle strength). We know from our prior work, the number of animals that would be required to demonstrate this difference (if a drug is effective) with a high likelihood of success. As such, the animal numbers proposed here ensure that their ethical use is maximised because we will use sufficient numbers of animals to ensure success of our experiments, but avoid use of more animals than are needed.

The majority of the healthy animals generated in this project are rehomed as pets and a minority are used for ongoing research.

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

We use the results of previous work, and where possible, stored tissues from previous studies, further to reduce the number of animals required. Experiments are typically conducted according to ARRIVE (2.0) guidelines and design is examined and scrutinised closely by external or professional statisticians and a separate Scientific and Ethical Advisory Board who are independent from the researchers to ensure that the proposed work is valid and ethical. This is an additional level of scientific and ethical scrutiny that occurs beyond normal regulation. The NC3Rs Experimental Design Assistant will be used for algorithm-generated feedback on adjustments that could be made (such as identifying potential sources of bias/nuisance variables) and, where appropriate in representing experimental design visually for group or external discussion. We utilise other online resources (such as GLIMMPSE) to ensure that numbers of animals used will maximise the chance of a positive outcome, when using repeated measurements, to increase the power of our statistical comparisons.

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

We will share results of animal use between studies where possible and in the course of this work will continue to generate a database of results that can be used as historical information to enable us to compare different treatments. Our goal is to generate a complete online dataset that can be openly accessed for historical natural history data from this colony so it becomes a resource for other researchers.

A retrospective assessment of reduction will be due by 28 March 2028

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

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 un[illegible]Desing bon

In addition, work conducted within our facility, in conjunction with BSU staff, is performed with attention to the Culture of Care, promoted by the PREPARE guidelines to which we espouse in ongoing work and in particular, when planning and preparing future projects.

How will you stay informed about advances in the 3Rs, and implement these advances effect]