Home Office

NON-TECHNICAL SUMMARY

Breeding and therapy of the DE50-MD dog model of Duchenne Muscular Dystrophy

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
- (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

Duchenne Muscular Dystrophy, Therapy, Animal model, Dog, Genetic disease

Animal types	Life stages
DE50-MD Beagle cross	juvenile, neonate, adult, embryo, pregnant, aged

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Uses cats, dogs or equidae

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?

This project aims to maintain a colony of Beagle cross dogs (DE50-MD) with naturally-occurring muscular dystrophy that will be used to test promising therapies prior to clinical trials in human patients with Duchenne muscular dystrophy (DMD).

A retrospective assessment of these aims will be due by 28 March 2028

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

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?

DMD is a disease first recognised in young boys caused by mutations in an X-linked gene that is critical for muscle function but that also causes behavioural and cognitive problems. Sufferers are confined to a wheelchair by the age of 12, are effectively paralysed by their 20s and all die in their late 20s or early 30s due to progressive wasting of all muscles including the heart and in particular, respiratory muscle failure. The disease is the most common genetic disorder diagnosed in childhood and has a worldwide prevalence of 1 in 3600-5000 male births. Optimal medical management has improved quality of life and increased lifespan from an original age of death at 16, but can do little to prevent the relentless muscle wasting. Pet dogs also are affected with this condition, so our work will also benefit pet dogs and their owners in the future.

The development of treatments for this condition mostly relies on cells in culture and the mdx mouse model of the disease. The mdx mouse is a good biochemical model but does not show the clinical signs typical of the disease in boys and the immune system in mice differs to that of humans such that responses to (for example) gene therapy viral vectors can be very different between these species. Consequently, there is doubt about the ability to directly translate results in the mouse into a human

clinical trial. In contrast, dystrophic dogs show similar clinical and pathological progression to humans and have similar immune responses, so can serve as a final test to enable rational decisions about which treatments are most likely to be successful in humans.

In this programme, we will test treatments (such as gene therapies) planned for boys with DMD and we will also be examining the optimal way to deliver these treatments, comparing, for example, whether a sustained administration over several hours is better than a single short dose of a treatment.

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

This work is to be conducted with a primary aim of finding and optimising effective treatments or a cure for Duchenne Muscular Dystrophy. We will investigate novel treatments (such as gene therapies) or ancillary treatments, publish the results and promote the licensing of drugs that are to be taken into human clinical trials.

A secondary aim of this work is to provide much needed information (via publication) regarding the underlying disease mechanisms at play in Duchenne Muscular Dystrophy. A species comparison approach (for example between mice, dogs and humans) with the same disease is a powerful method by which to examine conserved disease mechanisms, which themselves can then be a target for future therapeutic investigations.

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

The work will be of primary benefit to human patients with Duchenne Muscular Dystrophy and their families and carers. Furthermore, the work will have a secondary benefit to society because the emotional and financial costs for dealing with this currently fatal disorder are very substantial.

The work has secondary benefits in other diseases because many of the approaches we plan to take can be applied in other disorders.

An additional longer term benefit might be in treatment of the same condition in pet dogs in the future, thereby benefiting the animal and the owner.

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

In all our work to date we have promoted our findings (via the BBC, national press, in patient led meetings). We will discuss our work at domestic and international research meetings and publish our work in high impact, peer reviewed research journals under an open access policy so that it is available to everyone.

We recognise that it is important to publish negative findings in order to prevent other researchers repeating our work. We will do this as well.

Species and numbers of animals expected to be used

- Other dogs: No answer provided

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 different to those that might be conducted on pet dogs during investigation of disease by qualified veterinary surgeons. Animals will undergo non- or minimally-invasive procedures such as blood sampling, ultrasound and clinical examinations. Some dogs will undergo procedures (such as muscle biopsy and MRI) under general anaesthesia, but we will follow the same procedures that are conducted routinely by vets in pet dogs. Some dogs will undergo functional assessments to measure their muscle strength by stimulating muscle contraction whilst under anaesthesia and by examining activity and walking, by videoing and through the non-invasive use of activity monitors on the dogs' collars.

All dogs have access to grassy paddocks and are kept in groups (except when whelping) to enhance their welfare. They have daily human interaction and are assessed daily for their welfare. They are fed the same as pet dogs.

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

We do not expect any adverse effects of the therapies or research that we are conducting. All therapies will have first been tested in cell culture and/or in rodent or other animal models. Of the procedures that we are performing, muscle biopsy is associated with mild discomfort in people that can readily be controlled with pain relief. We will routinely use pain relief in our dogs whenever needed or other

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. Muscular Dystrophy in humans is not associated with pain. As in humans, affected dogs become weaker as the disease progresses and sometimes the dogs can have problems with swallowing, so we carefully monitor these aspects in particular and have defined humane endpoints so that these problems do not compromise the welfare of the animal.

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)?

The majority of animals that will be covered by this licence will be categorised as 'subthreshold' or 'mild' as these are breeder animals and unaffected littermates which are then rehomed. Breeding of affected dogs with Duchenne Muscular Dystrophy is categorised as 'moderate' due to their genetic disease and to the minor surgical procedures that they undergo. Up to 90 animals will be in this category.

We maintain typically no more than 12 carrier female dogs of breeding age and up to 3 normal male adult stud dogs. Of the puppies that are born, 75% of animals are either normal or carrier animals. 25% of puppies are affected male puppies.

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

- Killed
- Kept alive
- Rehomed

A retrospective assessment of these predicted harms will be due by 28 March 2028

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

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?

Animal studies are required because of the complex disease processes that occur in humans with this disease that cannot recapitulated fully in cell culture. Because mice do not display clinical signs of Duchenne Muscular Dystrophy, we cannot assess response to treatment in mice, and instead need to study a model that displays the weakness and muscle problems that are seen in humans.

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 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.

Whilst other dog models of Duchenne Muscular Dystrophy are used in research, the model we use (unlike others) has a naturally occurring mutation that is in the region of the dystrophin gene that is most commonly mutated in humans. This means that this model is more applicable to several of the most promising treatments currently being evaluated. In addition, the dog breed we use weighs less

than other dog models, and as a result, they are less affected by the muscle weakness that develops as they get older.

Generally the methods we use are similar or identical to those used by veterinary surgeons when investigating disease in pet dogs. Like them, we use pain relief medications for procedures that might cause discomfort.

We take welfare aspects of this project especially seriously. All dogs are socialised and have access to outside runs and paddocks to play with other animals and humans. We monitor the dogs very closely for signs of progression of their disease and make decisions to end the studies before the dogs reach the end stages of disease that occur in humans with this same disorder.

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

Mouse and fish models of Duchenne Muscular Dystrophy are used by other researchers to evaluate Duchenne Muscular Dystrophy and they are key components in the drug evaluation pathways. These animals however do not sufficiently reproduce the clinical disease features (muscle weakness) that occurs in this disease and they have immune systems that behave differently to those of humans. As such, these animals are often not suitable for functional efficacy testing of therapeutics or for studies for which the subject's immune system is relevant (such as gene therapies).

For our work, we are often interested in long term functional efficacy and safety of therapies, such that terminally anaesthetised animals are not suitable for the vast majority of procedures.

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

All animals used in our work are socialised through human interaction and we spend a lot of time training them (as with pet dogs) so they are familiar and accustomed with the procedures they undergo. Animals are maintained in groups and have access to outside runs and grassy paddocks and toys to run and play.

Any procedure that might be associated with discomfort is performed under local or general anaesthesia and we always provide pain relief drugs of the type used in pet dogs and people.

When we can, we minimise use of blood collection needles through use of a preplaced intravenous catheter, of the type that is used in pet dogs.

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

We will follow guidance generated by the NC3Rs in terms of husbandry: https://www.nc3rs.org.uk/3rs-resources/housing-and-husbandry-dog

All our work will be done in accordance with the ARRIVE 2.0 principles, and we always provide an ARRIVE statement in our publications confirming that this was done. We follow LASA guidelines whenever appropriate for administration routes and blood sampling protocols.

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 effectively, during the project?

We will conduct regular 3Rs assessments for our work and utilise online tools and information and advice from the NC3Rs including their Resource Library and Resource topics. We will communicate with our NC3RS liaison officer and institute changes whenever we can to improve animal welfare, reduce numbers of animals required and refine the methods.

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

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?