<table>
<tr><td>Project Title (max. 50 characters)</td><td>Testing therapies for neuromuscular diseases</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Muscular dystrophy, mdx mouse, gene therapy, oligonucleotides, muscle physiology.</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[1]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td>Yes</td><td></td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>There are currently no treatments for neuromuscular diseases that stop or reverse the decline in muscle function. Using mouse models we aim to develop clinically applicable therapies for these conditions via gene therapy or the use of drugs targeting disease symptoms.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>Neuromuscular diseases are an area of high unmet medical need that affect not only the individual patient but also their relatives and others involved in caring for the patient. Many cases of Duchenne muscular dystrophy (DMD) appear spontaneously with no family history, hence they cannot be effectively controlled by genetic counselling. DMD is debilitating and fatal and reducing or stopping the progression of the disease would be life-changing for patients and their carers.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>All work will be done in cell culture or in mice. Many of the assays in mice will be carried out under terminal anaesthesia or post-mortem. We expect to use less than 3,000 mice over the 5 project period.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of</td><td>The overall aim of the project is to develop clinically applicable therapies. Thus we seek to be as minimally invasive as possible. Most of the studies involve injections or addition of potentially therapeutic drugs to food or water and no surgical procedures except under terminal general anaesthesia (when the mouse does</td></tr>
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[1] Delete Yes or No as appropriate.
[2] At least one additional purpose must be selected with this option.

severity? What will happen to the animals at the end?	not feel pain or wake up at the end). As such, most of the procedures are of only mild severity. However, some of the work with cell based therapies (less than 200 mice) will require recovery surgery and this will be of moderate severity.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In the vast majority of cases the drugs and genetic constructs under test will have been evaluated in cell culture. However, it is not possible to fully evaluate the treatment effects without testing in an intact whole animal with functional nervous and hormonal regulation of cellular processes and the complex inter-relationship of the muscle or brain and spinal cord and the blood supply which may act to limit drug effectiveness.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use the standard operating procedures (currently located on the treat-NMD website: http://www.treat-nmd.eu/research/preclinical/preclinical-efficacy-standards/). We have considerable experience with the mdx mouse model which provides knowledge of the variation in each measure and therefore accurate calculations of the required sample size. Experiments will use a randomised block design in most cases where mice in the same litter are assigned to different treatments at random to compensate for any litter to litter effects. Where the effect of a specific intervention is unpredictable and the potential variation is uncertain, pilot trials using 3 animals per group with a limited number of groups will be used to assess the variation before larger scale experiment using a range of doses with group sizes determined using power calculations. In some cases it may be possible to use a special method (factorial design) that reduces group sizes to 3-4 per group.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	All experiments will be conducted in mice as this species has been the most widely used for genetic manipulation and has the greatest number of spontaneous and induced mutants and genetically modified strains. It is also the lowest vertebrate group for which there are models of the most common neuromuscular disorders. The main model for the initial in vivo assessment of therapies for DMD will be the mdx mouse and this will be used for the majority of the studies: the mdx mouse model of DMD is a relatively mild model that shows no obvious signs of the disease. Most of the studies involve injections or addition of potentially therapeutic drugs to food or water and no surgical procedures except under terminal general anaesthesia (when the mouse does not feel pain). We will use anaesthesia

	and appropriate analgesia where a procedure may cause pain.