	Inventaris Wob-verzoek W17-07								
		wordt verstrekt				**weigeringsgronden**			
nr.	**document NTS2016801**	**reeds openbaar**	**niet**	**geheel**	**deels**	**10.1.c**	**10.2.e**	**10.2.g**	**11.1**
1	Aanvraagformulier				x		x		
2	Projectvoorstel				x		x	x	
3	Niet-technische samenvatting	x							
4	Bijlage beschrijving dierproeven 1				x		x	x	
5	Bijlage beschrijving dierproeven 2				x		x	x	
6	Bijlage beschrijving dierproeven 3				x		x	x	
7	Bijlage beschrijving dierproeven 1 aangepast				x		x	x	
8	Bijlage beschrijving dierproeven 2 aangepast				x		x	x	
9	Bijlage beschrijving dierproeven 3 aangepast				x		x	x	
10	DEC-advies				x		x	x	
11	Ontvangstbevestiging				x		x		
12	Verzoek om nadere aanvulling CCD				x		x	x	
13	Verzoek om nadere aanvulling CCD - email 1				x		x	x	
14	Verzoek om nadere aanvulling CCD - email 2				x		x	x	
15	Reactie op verzoek om nadere aanvulling CCD				x		x	x	
16	Advies CCD		x						x
17	Beschikking en vergunning				x		x	x	

AVD 1030020168 01

27 DEC. 2016

Aanvraag
Projectvergunning Dierproeven
Administratieve gegevens

- U bent van plan om één of meerdere dierproeven uit te voeren.
- Met dit formulier vraagt u een vergunning aan voor het project dat u wilt uitvoeren. Of u geeft aan wat u in het vergunde project wilt wijzigen.
- Meer informatie over de voorwaarden vindt u op de website www.zbo-ccd.nl of in de toelichting op de website.
- Of bel met 0900-2800028 (10 ct/min).

1 Gegevens aanvrager

1.1 Heeft u een deelnemernummer van de NVWA?
Neem voor meer informatie over het verkrijgen van een deelnemernummer contact op met de NVWA.

☑ Ja > *Vul uw deelnemernummer in* 10300

☐ Nee > *U kunt geen aanvraag doen*

1.2 Vul de gegevens in van de instellingsvergunninghouder die de projectvergunning aanvraagt.

Naam instelling of organisatie	Stichting Katholieke Universiteit Nijmegen
Naam van de portefeuillehouder of diens gemachtigde	Instantie voor dierenwelzijn
KvK-nummer	4 1 0 5 5 6 2 9

1.3 Vul de gegevens van het postadres in.
Alle correspondentie van de CCD gaat naar de portefeuillehouder of diens gemachtigde en de verantwoordelijke onderzoeker.

Straat en huisnummer	Geert Grooteplein 10
Postbus	9101, t.a.v. [redacted]
Postcode en plaats	6500HB Nijmegen
IBAN	NL90ABNA0231209983
Tenaamstelling van het rekeningnummer	UMC St Radboud

1.4 Vul de gegevens in van de verantwoordelijke onderzoeker.

(Titel) Naam en voorletters	☐ Dhr. ☑ Mw. [redacted]
Functie	[redacted]
Afdeling	[redacted]
Telefoonnummer	[redacted]
E-mailadres	[redacted]

1.5 *(Optioneel)* Vul hier de gegevens in van de plaatsvervangende verantwoordelijke onderzoeker.

(Titel) Naam en voorletters	☑ Dhr. ☐ Mw. [redacted]
Functie	[redacted]
Afdeling	[redacted]
Telefoonnummer	[redacted]
E-mailadres	[redacted]

1.6	*(Optioneel)* Vul hier de gegevens in van de persoon die er verantwoordelijk voor is dat de uitvoering van het project in overeenstemming is met de projectvergunning.	(Titel) Naam en voorletters	[redacted] ☐ Dhr. ☐ Mw.
		Functie	Instantievoor Dierenwelzijn
		Afdeling	[redacted]
		Telefoonnummer	[redacted]
		E-mailadres	[redacted]
1.7	Is er voor deze projectaanvraag een gemachtigde?	☑ Ja > *Stuur dan het ingevulde formulier Melding Machtiging mee met deze aanvraag*	
		☐ Nee	

2 Over uw aanvraag

2.1	Wat voor aanvraag doet u?	☒ Nieuwe aanvraag > Ga verder met vraag 3
		☐ Wijziging op (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn Vul uw vergunde projectnummer in en ga verder met vraag 2.2
		☐ Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn Vul uw vergunde projectnummer in en ga verder met vraag 2.3
2.2	Is dit een *wijziging* voor een project of dierproef waar al een vergunning voor verleend is?	☐ Ja > Beantwoord dan in het projectplan en de niet-technische samenvatting alleen de vragen waarop de wijziging betrekking heeft en onderteken het aanvraagformulier
		☐ Nee > Ga verder met vraag 3
2.3	Is dit een *melding* voor een project of dierproef waar al een vergunning voor is verleend?	☐ Nee > Ga verder met vraag 3
		☐ Ja > Geef hier onder een toelichting en ga verder met vraag 6

3 Over uw project

3.1	Wat is de geplande start- en einddatum van het project?	Startdatum	22-01-2017
		Einddatum	22-01-2022
3.2	Wat is de titel van het project?	Development of a cell therapy against the muscular phenotype of Myotonic Dystrophy	
3.3	Wat is de titel van de niet-technische samenvatting?	Ontwikkeling van een celtherapie voor Myotone Dystrofie type 1	
3.4	Wat is de naam van de Dierexperimentencommissie (DEC) aan wie de instellingsvergunninghouder doorgaans haar projecten ter toetsing voorlegt?	Naam DEC	RU DEC
		Postadres	Postbus 9101, 6500 HB Nijmegen [redacted]
		E-mailadres	[redacted]

4 Betaalgegevens

4.1 Om welk type aanvraag gaat het?

☑ Nieuwe aanvraag Projectvergunning € 1.441,00 Lege

☐ Wijziging € Lege

4.2 Op welke wijze wilt u dit bedrag aan de CCD voldoen.
Bij een eenmalige incasso geeft u toestemming aan de CCD om eenmalig het bij 4.1 genoemde bedrag af te schrijven van het bij 1.2 opgegeven rekeningnummer.

☐ Via een eenmalige incasso

☑ Na ontvangst van de factuur

5 Checklist bijlagen

5.1 Welke bijlagen stuurt u mee?

Verplicht

☒ Projectvoorstel

☒ Niet-technische samenvatting

Overige bijlagen, indien van toepassing

☐ Melding Machtiging

☒ DEC-advies en factuurinformatie

6 Ondertekening

6.1 Print het formulier uit, onderteken het en stuur het inclusief bijlagen via de beveiligde e-mailverbinding naar de CCD of per post naar:

Centrale Commissie
Dierproeven
Postbus 20401
2500 EK Den Haag

Ondertekening door de instellingsvergunninghouder of gemachtigde (zie 1.6). De ondergetekende verklaart:

- dat het projectvoorstel is afgestemd met de Instantie voor Dierenwelzijn.
- dat de personen die verantwoordelijk zijn voor de opzet van het project en de dierproef, de personen die de dieren verzorgen en/of doden en de personen die de dierproeven verrichten voldoen aan de wettelijke eisen gesteld aan deskundigheid en bekwaamheid.
- dat de dieren worden gehuisvest en verzorgd op een wijze die voldoet aan de eisen die zijn opgenomen in bijlage III van richtlijn 2010/63/EU, behalve in het voorkomende geval de in onderdeel F van de bijlage bij het bij de aanvraag gevoegde projectvoorstel gemotiveerde uitzonderingen.
- dat door het ondertekenen van dit formulier de verplichting wordt aangegaan de leges te betalen voor de behandeling van de aanvraag.
- dat het formulier volledig en naar waarheid is ingevuld.

Naam	[redacted]
Functie	Instantie voor dierenwelzijn
Plaats	Nijmegen
Datum	22-12-2016
Handtekening	[redacted]

Form
Project proposal

- This form should be used to write the project propsal of animal procedures.
- The appendix 'description animal procedures' is an appendix to this form. For each type of animal procedure, a separate appendix 'description animal procedures' should be enclosed
- For more information on the project proposal, see our website(www.zbo-ccd.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10300
1.2	Provide the name of the licenced establishment.	Stichting Katholieke Universiteit Nijmegen
1.3	Provide the title of the project.	Development of a cell therapy against the muscular phenotype of Myotonic Dystrophy type 1

2 Categories

2.1 Please tick each of the following boxes that applies to your project.

[X] Basic Research
[X] Translational or applied research
[] Regulatory use of routine production
[] Research into environmental protection in the interest of human or animal health or welfare dier
[] Research aimed at preserving the species subjected to procedures

[] Higher education or training
[] Forensic enquiries
[] Maintenance of colonies of genetically altered animals not used in other animal procedures

3 General description of the project

3.1 Background

Describe the project (motivation, background and context) with respect to the categories selected in 2.

- For legally required animal procedures, indicate which statutory or regulatory requirements apply (with respect to the intended use and market authorisation).
- For routine production, describe what will be produced and for which uses.
- For higher education or training, explain why this project is part of the educational program and describe the learning targets.

Myotonic dystrophy (dystrophia myotonica, DM1 in short) is the most common adult form of muscular dystrophy, with a prevalence of approximately 10 per 100,000 people. This chronic progressive multisystemic disorder is characterized by cognitive problems, myotonia and muscular dystrophy with progressive muscle wasting leading to increased limitations in motor capacity. The progressive skeletal muscle phenotype represents one of the most disabling aspects of DM1. Muscle degeneration is followed by myofiber regeneration as the body has self renewal potency. The process of self renewal is initiated by resident [redacted] in the skeletal muscle. However, this is a limited system that is rapidly exhausted. Simulating the process of self renewal [redacted] complementing the defective muscle cells and fibers, is a highly promising approach to combat the muscular characteristics of DM1. We wish to study this approach, summarized in the figure below.

When using [redacted] we need to consider the [redacted] of DM1 present in [redacted]. The [redacted] As a consequence, cell metabolism is disturbed [redacted]

This disease causing process can be modeled in transgenic mice. We will use two mouse models ([redacted] from the [redacted] line. In the [redacted] mouse the [redacted]. In the [redacted] mouse model the [redacted], as a result of [redacted] transgenic mice carrying [redacted] with the [redacted] locus [redacted] show that muscle atrophy is already present at [redacted]. Maximal force generation is reduced with 35% in the skeletal muscles of [redacted]. This progressive weakness observed in these mice is directly related to the reduced muscle mass and muscle fiber size. No symptoms are found in the mouse model with a [redacted]

Research so far has been directed against the molecular effects of [redacted]. For example the degradation of [redacted] by [redacted]) or inhibition of protein binding. However, these approaches do not eliminate the causative [redacted]. [redacted]) can be used to remove the [redacted], thereby eliminating the presence of the [redacted]. Via this process healthy [redacted] with therapeutic value can be generated.

We will use two parallel approaches to [redacted]: (1) [redacted] tissue will be taken from the DM1 mouse model and healthy control mice to [redacted] and (2) patient and control [redacted] will serve to [redacted]. These [redacted] can be [redacted] by the [redacted] technique. Delivery of [redacted] coupled with [redacted], already resulted in [redacted] in cells from a DM1 patient and in cells from DM1 mice ([redacted]). In the next step, we would like to investigate whether [redacted] can be [redacted] in mice and contribute to muscle regeneration *in vivo*. We intend to use both [redacted] and [redacted] as well as [redacted] (and possibly [redacted] cells, see below) for their potential to participate to muscle tissue and muscle function in vivo. We have chosen for the parallel [redacted] cell approach since experiments in DM1 mouse models will provide a functional read-out where the effectiveness of the therapy can be measured, while [redacted] of [redacted] for the [redacted] will provide information on feasibility later on in humans. Answers to both the functional and the species aspect are in our view crucial to reach the goal of our project. A CMO (Commissie Mensgebonden Onderzoek) application has recently been approved by the [redacted]. We aim to collect [redacted] biopsies of nine DM1 patients and two healthy individuals to [redacted] and [redacted] which can be tested in [redacted] as shown in the illustration.

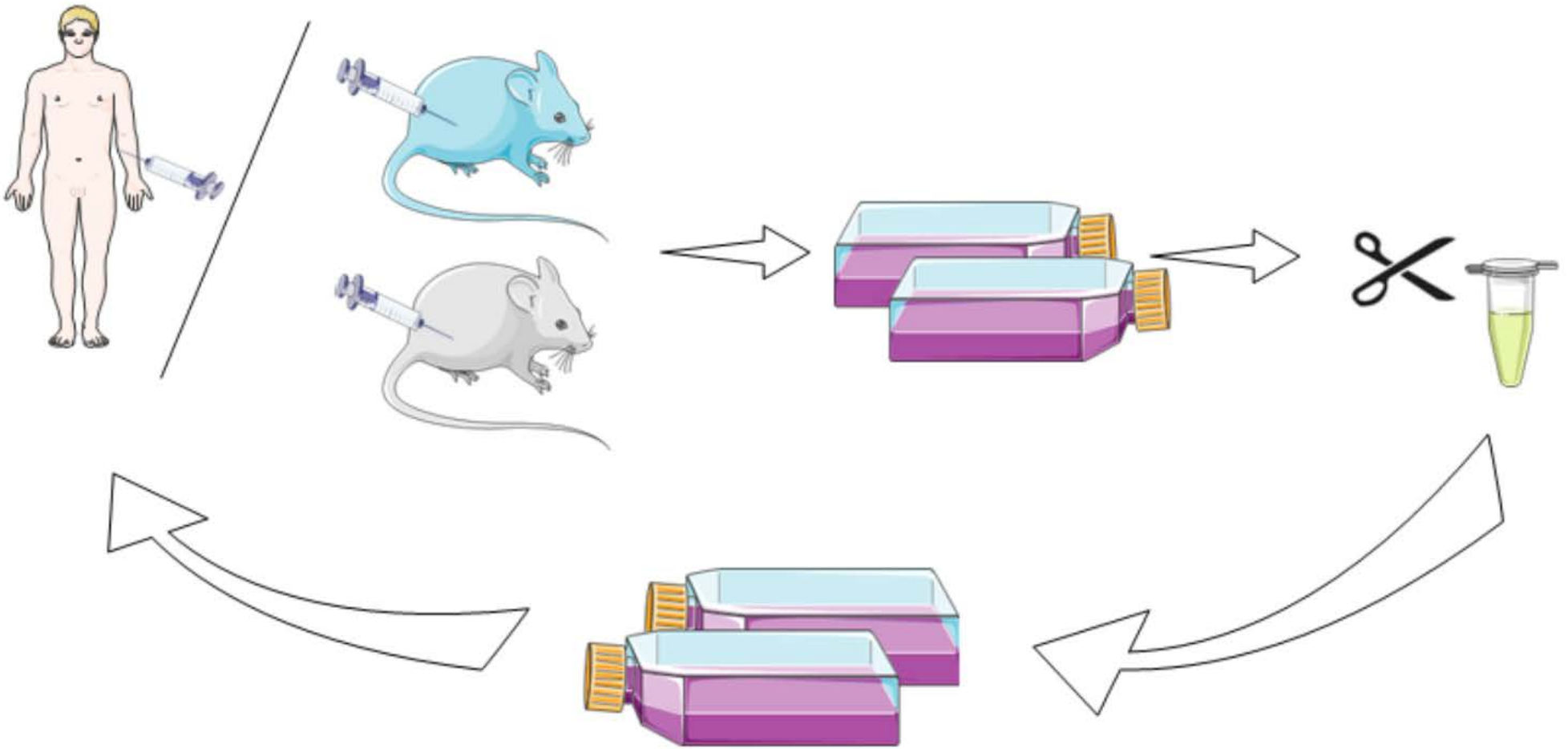
[redacted] are [redacted] [redacted] that form [redacted] (also known as a [redacted]). These [redacted] form [redacted] by the process of [redacted] are [redacted] [redacted] that can become [redacted] and have the potential to contribute to [redacted] The potential of both [redacted] and [redacted] has already been studied in the treatment of [redacted] [redacted] meet all criteria to be used as [redacted] They exhibit high [redacted] capacity, good *ex vivo* proliferation capacity and they have the capability to [redacted] thereby [redacted]. These properties have also prompted investigations towards the use of [redacted] in the treatment of [redacted]. Moreover, very recently a phase I/II clinical study started treatment with delivery of [redacted] boys. Transplantation of [redacted] in humans proved to be feasible and relatively safe ([redacted]

When we translate this approach to the clinic, [redacted] biopsy can be [redacted] and used as [redacted] therapy for muscle regeneration. This would be a preferred approach as we can easily obtain [redacted] and there is no immune reaction to the [redacted]. However, we need to be able to generate enough [redacted] [redacted] [redacted] for therapy purposes. There are a few variables to take into account: i) the number of [redacted]; ii) the number of [redacted] that can be [redacted]; and iii) the amount of [redacted] for [redacted] depending on the number of [redacted] that home to the [redacted] place and contribute to myogenesis. If we need more [redacted] than we can generate via [redacted] in [redacted] [redacted] we will use [redacted] from DM1 patients. This approach has been used

before in research to [redacted] are generated from somatic cells of DM1 patients. The [redacted] represent an [redacted] of cells that can be differentiated towards [redacted] lineages such as [redacted] and [redacted] By injecting [redacted]-derived [redacted] [redacted] into an [redacted] mouse model ([redacted]) we can investigate the contribution to regeneration of skeletal muscle.

In sum, in this project we aim to [redacted] from DM1 mouse models and patients (i.e., [redacted] or [redacted] in which the [redacted]. These [redacted] [redacted] [redacted] will be tested on their therapeutic value after administration into mice.

[redacted] [redacted] [redacted]



[redacted]

3.2 Purpose

Describe the project's main objective and explain why this objective is achievable.

- If the project is focussed on one or more research objectives, which research questions should be addressed during this project?
- If the main objective is not a research objective, which specific need(s) does this project respond to?

In this project, we propose a preclinical study aimed to develop a new strategy for therapeutic intervention against the [redacted] phenotype in DM1. [redacted] and [redacted] have the potential to functionally integrate in [redacted] and contribute to [redacted]. Therefore, we aim to investigate whether [redacted] and [redacted] or [redacted] can be used for regenerative [redacted] therapy. These objectives are within reach as:

a.) [redacted] of [redacted] from mouse [redacted] has been achieved before ([redacted]). During a stay of 1 month in the lab of [redacted] gained experience in [redacted] of [redacted] The group of [redacted] published on [redacted] and therapeutic approaches for DMD and is considered to be the leading research group in the field ([redacted]). We are now in the process of setting up the pericyte isolation techniques in our own lab, here in Nijmegen.

b.) the technique to [redacted] the [redacted] is present in our lab and is succesful in [redacted] and [redacted] cells ([redacted]

c.) delivery of [redacted] has been performed previously for treatment of [redacted]

d.) [redacted] was shown to be a promising therapeutic approach in patients with [redacted] The group has ample experience working with [redacted] and [redacted] In addition, a collaboration exists with [redacted] and very experienced with [redacted] in [redacted] (also called [redacted]

3.3 Relevance

What is the scientific and/or social relevance of the objectives described above?

DM1 is a complex, [redacted] and [redacted] disorder with one of the most [redacted] clinical pictures. Despite the huge impact of DM1 on daily life of both patients and their family members, DM1 patients fail to receive the quality of healthcare that is, or should be, available. Often they are not assertive users of the health care system. Several treatments are focused on reducing limitations and supporting participation in everyday activities. Large efforts are recently set up to improve quality of life of DM1 patients by internationally improving clinical practice and standards of care. For example, the [redacted]) aims to use exercise therapy and Cognitive Behavioural Therapy (CBT)

to improve functional capacity, improve muscle function, stimulate an active lifestyle, reduce fatigue sensation and increase quality of life [redacted]

There is currently no cure nor treatment available for the [redacted] of DM1. In this project, we will contribute to better care for patients by testing the feasability of a [redacted] in mice by using [redacted]s. We will a) determine the optimal conditions for [redacted] b) establish an efficient protocol for [redacted] of the [redacted] and c) determine the most optimal conditions for [redacted]. Finally, we will examine the contribution of [redacted] to [redacted] by investigating how [redacted]. Taken together, this project will result in valuable novel knowledge that contributes to a better understanding of the important and therapeutic role [redacted] play in patients with [redacted] disorders.

3.4 Research Strategy

3.4.1 Provide an overview of the overall design of the project (strategy).

Aim 1: [redacted]
Aim 2: [redacted]

Approach:
Obtain [redacted]
- mouse [redacted]
- mouse [redacted]
- [redacted]
- human [redacted]
- human [redacted]

To [redacted], mice will be sacrificed by cervical dislocation or CO2 euthanization and [redacted] (usually from the hind leg) are dissected. Human [redacted] are isolated from a skeletal muscle biopsy of participants (CMO application; approved), human DM1 patient or control [redacted]) are already available in our lab. [redacted] will be purchased. [redacted] methodology has already been applied in our lab on [redacted] from DM1 mice and on [redacted] from a DM1 patient [redacted] In short, the procedure is as follows: cells are [redacted] expressing [redacted] and [redacted] The [redacted] with a [redacted] allows us to study the effectiveness of transduction. We will optimize this entire process to obtain:

a) the most [redacted] protocol (number of [redacted] obtained [redacted]),
b) highest percentage of [redacted] with [redacted],
c) the maximum number of [redacted] by the maximum amount of amplification/passages.

We want to investigate the ability of the [redacted] mouse [redacted] to contribute to functional [redacted] To assess *in vivo* [redacted] of [redacted] and wild-type healthy mouse [redacted] we will [redacted] in DM1 mice or [redacted] litter mates, preferably in the hind leg muscle. For the experiments we will [redacted] (either by [redacted] or placebo (salt solution). To investigate the feasibility of this therapeutic approach in [redacted] (obligatory preclinical research), [redacted] are [redacted] and [redacted] into [redacted] These [redacted] show no [redacted] phenotype, therefore a functional read-out is not possible but the feasibility question will be answered. [redacted] represent an infinite source of cells that can be differentiated towards [redacted] lineages such as [redacted] and [redacted] Because of the unlimited number, these cells are valuable for additional experiments e.g. [redacted] can provide information of the effect of the [redacted], proliferation, differentiation, homing and myogenic capacity. They can be used to optimize [redacted] and if the number of [redacted] and/or genetically corrected from patients/mouse models is too low or the amount of cells needed for functional recovery is too high, we plan to use unlimited hiPSC.

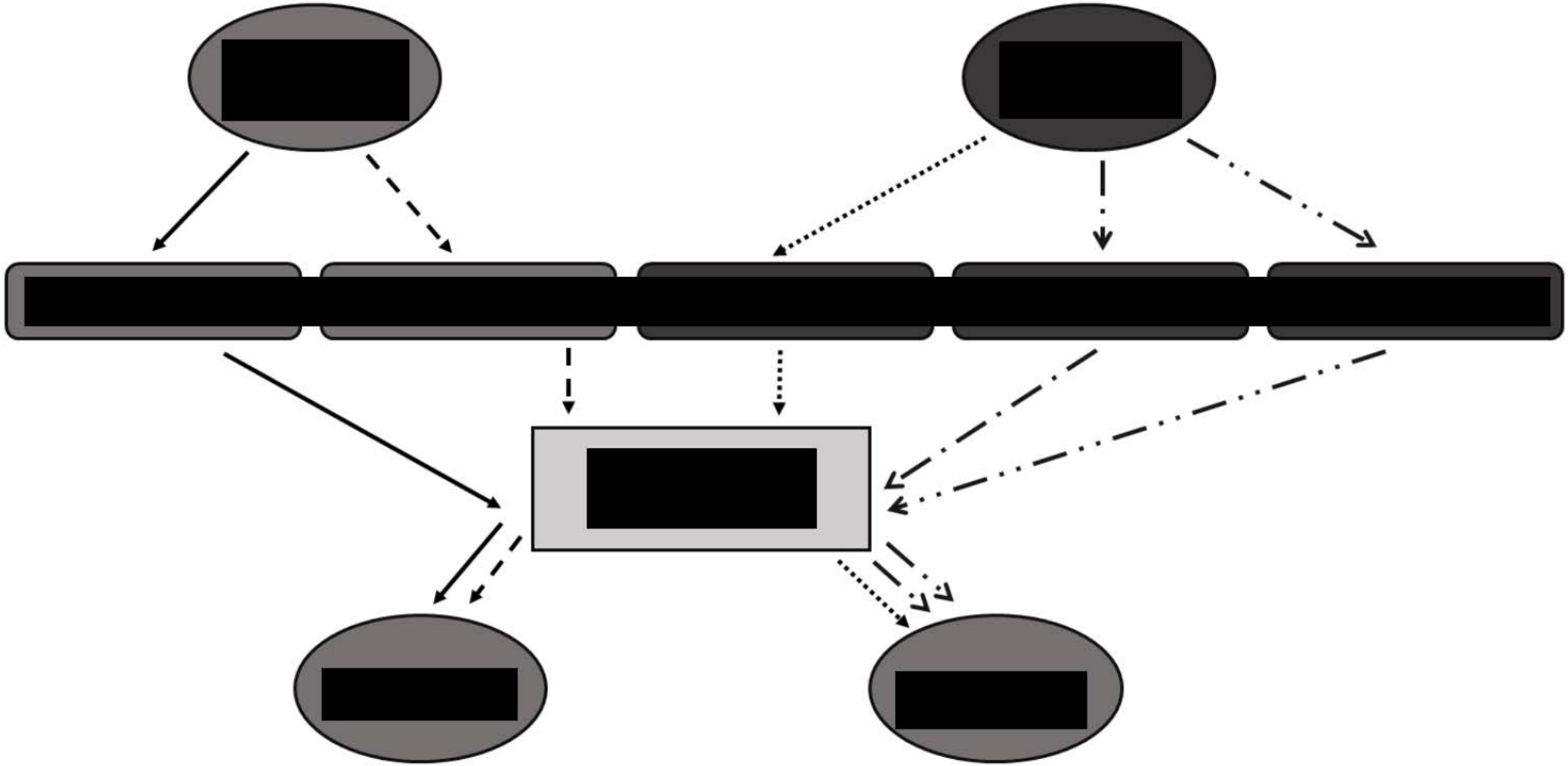
After [redacted] mice will be sacrificed and [redacted] will be collected for molecular or immunohistochemical analysis or for functional analysis of [redacted] *in vitro*. The number of [redacted] that have successfully engrafted and survived are analyzed. This way we can analyze the competition between [redacted] and [redacted] and investigate the differences in differentiation or contribution to [redacted] In addition, we can trace the localization and see whether [redacted] have homed to other tissues, e.g. the vascular system and fat bodies. The similar identity in [redacted] and the immune-privileged properties of the [redacted] will preclude immune responses against the [redacted]. To enhance [redacted] uptake of the cells we may [redacted]. Based on the outcome of these initial experiments we will vary [redacted] conditions [redacted]. The starting [redacted] conditions are based on experiences from the lab of M. Sampaolesi. *In vitro* analysis of muscle (fiber) function in a tissue bath can be done as described by for example Park et al (J. Vis Exp. 2012).

The final experiments in DM1 mouse models are focused on assessing the functional incorporation of the [redacted] in [redacted] tissue after [redacted] We will compare [redacted] with placebo-treated animals. Functional measurements will be performed to [redacted]. These parameters can be assessed by a [redacted]

In conclusion, we want to show that:
1. We can [redacted].
2. [redacted].
3. [redacted].

3.4.2 Provide a basic outline of the different components of the project and the type(s) of animal procedures that will be performed.

The general outline is presented in the figure below. From DM1 mice, [redacted] or [redacted] will be [redacted] which will be [redacted] After removal of the [redacted] the functional potential of the [redacted] is established by administration of the [redacted] into [redacted] mice. Moreover, in parallel, [redacted] and [redacted] Administration of these cells into immunocompromised ([redacted] mice enables us to study their therapeutic potential. An important benefit of [redacted] is the unlimited source. Many [redacted] can be generated from patient and control somatic cells.



Generation of [redacted] models

- General: weigh animals, 2x a week for [redacted] mice or [redacted] mice.
- Two DM1 mouse models are bred from the [redacted] line, the [redacted] and [redacted] models, which differ in the length of the ([redacted] in the [redacted] mice show a relatively high [redacted] and will only be born from [redacted] crossings (breed with discomfort).

[redacted] and [redacted] of DM1 and [redacted] mouse cells
- Sacrifice [redacted] day old DM1 and [redacted] mice
- Dissection [redacted]
- [redacted] of [redacted] or [redacted] from [redacted]
- [redacted] of [redacted] with [redacted]
- Amplification [redacted]

Purchase [redacted] and differentiation towards [redacted] lineage
- Purchase and validate [redacted] from DM1 patients
- Set up [redacted] differentiation protocols towards [redacted] and [redacted]

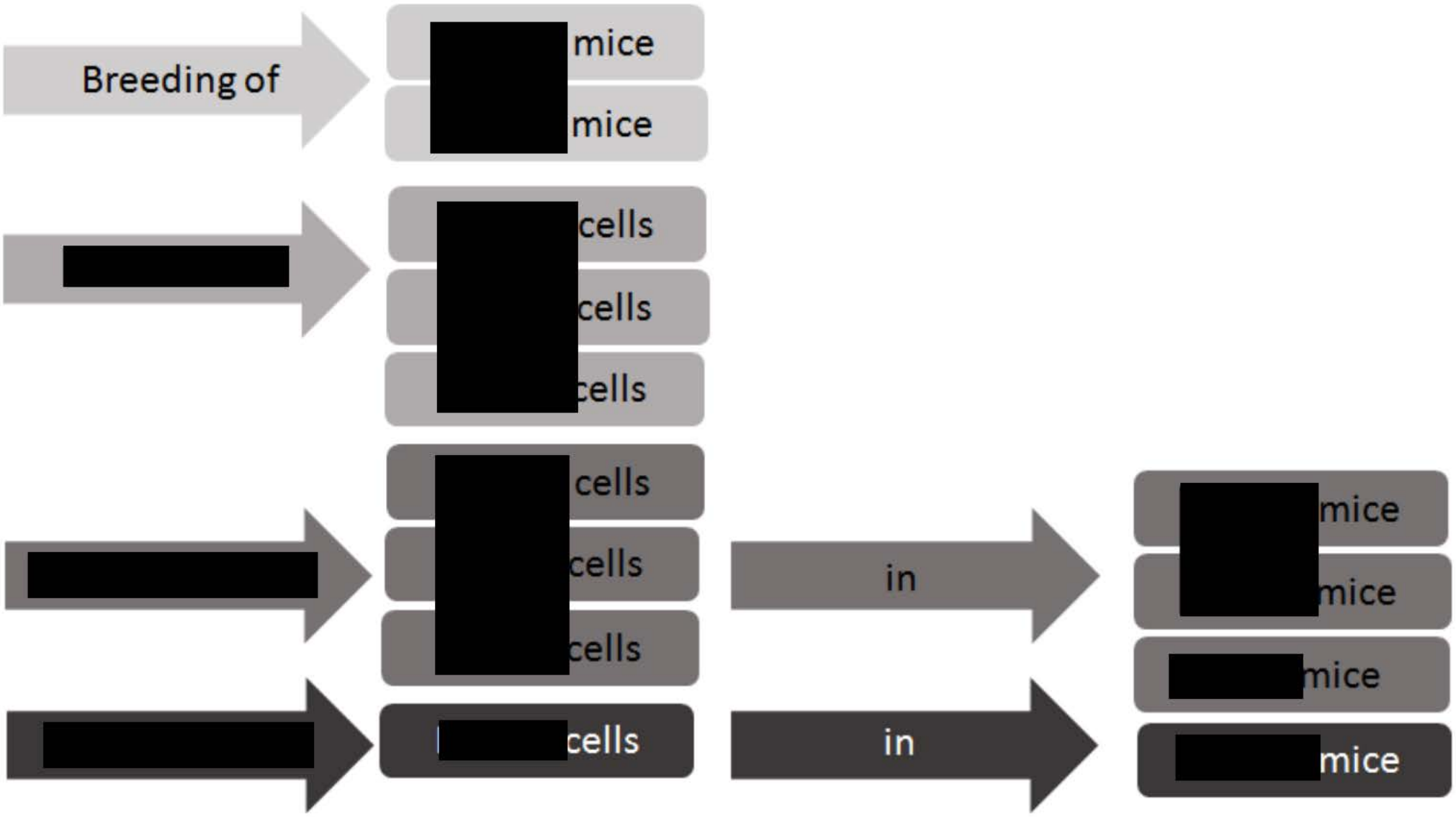
[redacted] and [redacted] of human [redacted] and [redacted] (CMO application; approved)
- [redacted] is performed from upper leg of patients/controls
- [redacted] of [redacted] or [redacted] from dissected muscle
- [redacted] of [redacted] with [redacted]
- Amplification isolated progenitor cells

[redacted]) application cells ([redacted] vs. saline solution
- [redacted] with [redacted] cells/vehicle in mice (DM1 mice for mice [redacted] for human [redacted]
- One leg is treated with a [redacted], the [redacted], while the other leg receives a saline solution. We will start with 1 [redacted] per leg per day per mouse up to max. 5 [redacted]. Max. 5 [redacted] days per week. Max. 20 [redacted] per mouse. The [redacted] of the other leg serves as a control. The exact final protocol of treatment will be based on the outcome of a small pilot study. All experiments will be conducted by art. 9/12 researchers who will minimize discomfort as much as possible. For example, to counteract suffering early enough, animals will be regularly observed and weighed. The animal model has been used extensively by [redacted]. Animal handling and the planned procedures have been [redacted].
- *In vitro* analysis of [redacted]) function in a tissue bath can be done as described by for example Park et al (J. Vis Exp. 2012).
- Functional analysis ([redacted].
- Molecular and histological analysis (staining muscular proteins, PCR etc.).

[redacted]) application of [redacted]
- Mice are exposed to exercise to [redacted]. A single round of eccentric exercise a few hours prior to [redacted] damaged and inflammed muscle tissue (among others; increase IL, integrins, NF-kB signaling). Increase in inflammation markers can be measured in a blood sample taken before and after exercise.

- Starting with 1 injection per mouse up to max 5 injections. The exact final protocol of treatment will be based on the outcome of a small pilot study. Mock-injected mice will serve as control.
- Investigate functional effects of [redacted] e.g. by [redacted].
- Transcardial perfusion under anesthesia and isolation organs or euthanasia and isolation of organs for histological and functional analyses.
- Investigate homing to [redacted] and other tissues by microscopy

* For all approaches/protocols the exact final treatment will be based on the outcome of ongoing pilot studies. Choosing the most effective approach with minimal discomfort.



The figure represents a flowchart of the [redacted] in different models. [redacted] and [redacted] mice are bred. [redacted] from these models or from [redacted] from the [redacted] and [redacted] mice or human [redacted] will take place into [redacted] [redacted] and [redacted]. [redacted] is only performed with [redacted] into [redacted]

The rescue experiments with the [redacted] in the DM1 mouse models are the first experiments we will start with. When we are able to [redacted] and [redacted], they are expected to still have a high [redacted] and contribute to [redacted] regeneration. However, we are aware that this is a limited [redacted] Moreover, that these are mouse [redacted] For completion of the project including human DM1 patient or control [redacted], already available in our lab) and [redacted] from DM1 patients and control somatic cells is nescessary. As mentioned in 3.4.1.; if the number of [redacted] that can be [redacted] is too low or the amount of cells needed for functional recovery is too high, we plan to use [redacted] The benifit of these [redacted] is that [redacted] a.) represent an infinite source of cells, b.) can be differentiated towards [redacted], c.) these cells have a [redacted]. Moreover, because of the unlimited number these [redacted] are valuable for additional experiments e.g. validating [redacted], optimizing [redacted]. DM1 patient [redacted] or [redacted] [redacted] are then used to stimulate regeneration of [redacted]. Similar outcome measures are performed to determine therapeutic potential (molecular and histological analysis such as staining muscular proteins, PCR etc.).

3.4.3 Describe the coherence between the different components and the different steps of the project. If applicable, describe the milestones and selection points

Inclusion of [redacted] and [redacted] mice models:
[redacted] mice and [redacted] mice both display an increased [redacted] of respectively [redacted] mice display a longer [redacted] than [redacted] mice, and reproduce patient symptoms such as [redacted] weakness. The variability in [redacted] and variability in symptoms is also present in DM1 patients. To most optimally represent the DM1 patient population we want to include both [redacted] and [redacted] mice for [redacted] ánd [redacted]. This will provide valuable information as it will confirm that the therapeutic approach is applicable on a range of [redacted] and therefore a broad patient spectrum.

In addition, we expect differences between the [redacted] and [redacted] mice. Since the [redacted] mice represent a more [redacted] the presence of [redacted]and therefore the amount of [redacted]) might differ from the [redacted] mice. It has been communicated ([redacted] that dystrophic mice early in life present a higher percentage of [redacted]. Differences in functional recovery after [redacted] might also occur. We hypothesize that homing of [redacted] is better in [redacted] mice due to the signaling of the more [redacted] tissue, but that survival or contribution to [redacted] might be worse in this mice model due to the negative environment. These are all important aspects to consider in the context of the [redacted] observed in DM1. Including two DM1 mouse models therefore provides valuable information that cannot be collected with the inclusion of only one DM1 mouse model.

Coherence/ milestones:
Successful completion of each separate step/protocol as described in chapter "3.4.2 Outline" will provide the required information for the next research objective. When we are able to [redacted], we hypothesize that after [redacted], [redacted] [redacted] are able to contribute to [redacted] in [redacted] muscles. These promising results will take us to the next step of [redacted] We aim to stimulate [redacted] [redacted] by excessive exercise and start the trial with [redacted] to ameliorate myopathy.

3.4.4 List the different types of animal procedures. Use a different appendix 'description animal procedures' for each type of animal procedure.

Serial number	Type of animal procedure
1	3.4.4.1 [redacted] from [redacted]
2	3.4.4.2 [redacted] application of [redacted] or saline solution
3	3.4.4.3 [redacted] application of [redacted]

4

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website www.zbo-ccd.nl.
- Or contact us by phone. (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10300	
1.2	Provide the name of the licenced establishment.	Stichting Katholieke Universiteit Nijmegen	
1.3	List the different types of animal procedures. Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.	Serial number 1	Type of animal procedure 3.4.4.1 [redacted] of [redacted] from [redacted]

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

The overal aim is to investigate whether [redacted] ([redacted] and [redacted] can contribute to [redacted] Therefore DM1 mice are bred to 1.) [redacted] and 2.) to administer [redacted] and monitor improvements in [redacted] histology and phenotype.
Mice [redacted] of age will be sacrificed. We will dissect [redacted]. This will be done on the DM1 mouse model, a myotonic dystrophy mouse model, and wild-type littermates. The department [redacted] has experience with breeding of [redacted] and [redacted] mice [redacted]. We need to optimize the process of [redacted] to obtain a.) the most efficient generation protocol (number of [redacted]), b.) highest percentage of [redacted] with complete 'clean' [redacted] by [redacted], c.) the maximum number of [redacted] by the maximum amount of amplification/passages. Optimization of these steps will give valuable and useable information about the feasibility to translate the research into clinical practice.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

The DM1 mouse models with [redacted] are from the [redacted] line. To obtain [redacted] DM1 mice are sacrificed. Optimization of the procedure is established with [redacted] and [redacted] littermates born from [redacted] breeding. We will use CO2 euthanisation to sacrifise the animals as suggested by the protocol of [redacted] We can than easily perform [redacted] dissection. [redacted] tissue is cleaned from fat and tendon. Cells of interest are [redacted] characterized and [redacted] is applied for [redacted] This technique has already been applied in our lab for [redacted] from this DM1 mice model. In short, the procedure is as follows: cells are [redacted] with [redacted] and [redacted] by [redacted]. The [redacted] with a [redacted] allows us to study the [redacted]

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

With respect to the animal procedures; the [redacted] and [redacted] mice will be bred as previously described by our lab in [redacted]. Keeping the [redacted] line will only be done with [redacted] breeding, eliminating the birth of [redacted] mice with [redacted]. Only when experiments are planned breeding with [redacted] will start, resulting in [redacted] mice. Mice will be identified via genotyping as soon as possible. However, it is impossible to identify and euthanize [redacted] pups that will [redacted]. The size of the mice or nest size do not influence chance of survival. The [redacted] is shortly after birth, a time window where we cannot interrupt the nest as this can cause stress and might lead the mother to eat the pups, including the [redacted] that do have a [redacted] [redacted] The cause of [redacted] [redacted] in the [redacted] [redacted] mice is [redacted] and never seen in [redacted] or [redacted] pups. Children with [redacted] DM1 suffer from [redacted] during birth. Whether this [redacted] [redacted] is involved in the breeding of [redacted] mice remains undefined. The symptoms reported from adult [redacted] mice resemble the classic [redacted] phenotype instead of [redacted] [redacted]

We will start [redacted] procedures with small pilot experiments (n=3) based on published/communicated results from other [redacted] models to test the feasibility and further optimize the procedure before starting the preclinical study with statistical significance. By optimizing the process of [redacted] [redacted] and amplification we aim to generate the maximum number of [redacted] usable as [redacted]) from one [redacted] dissection. Thereby we minimize the number of mice needed for follow up [redacted] experiments. These optimalization experiments are performed on [redacted] mice and wild type littermates born from [redacted] breeding. With these approaches the number of mice is reduced.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

It is established that the [redacted] mouse line is a good animal model for preclinical studies on [redacted] phenotype [redacted] Moreover, the model is well known in the [redacted] research area [redacted]) and already in house and used in [redacted] studies before at the department of [redacted]. The [redacted] mice show [redacted] and increased [redacted] No muscle pain or discomfort has been reported in literature. From previous experience we know that [redacted] mice show normal cage behaviour in standard environment. Only when challenged differences appear between [redacted] mice and wild-type littermates. [redacted] [redacted] mice reproduce slight [redacted] weakness and [redacted] from [redacted] of age, when in a challenging environment. With the use of the [redacted] model we can perform functional read-outs. [redacted] mice and [redacted] both display an increased, but different [redacted]. The variability in [redacted] in the [redacted] and [redacted] mice is also present in DM1 patients. To most optimally represent the DM1 patient population we want to include both [redacted] and [redacted] mice for [redacted] ánd [redacted] [redacted] of [redacted] [redacted] This will provide valuable information as it will confirm that the therapeutic approach is applicable on a broad patient spectrum.

DM1 mice from [redacted] of age are used for dissection of [redacted] showed that effecient [redacted] can be performed after the first week of birth ([redacted]). Before this age [redacted] of [redacted] is possible. Older mice are expected to yield fewer [redacted] For [redacted] protocols, mice will be kept to a maximum age of [redacted]. After this age, [redacted] of [redacted] becomes difficult (personal communication with [redacted] [redacted] from [redacted] tissue are characterized and [redacted]. We prefer including DM1 models over using control/non-DM1 mice models since this approach represents the clinical situation. We aim to set up an [redacted] therapy using a patients [redacted] cells to stimulate [redacted].

We want to perform 25 [redacted] experiments on [redacted] mice. Research on different [redacted] mice models shows that [redacted] of [redacted] is most efficient by using [redacted] (pers. communication [redacted] lab). Unfortunately, [redacted] mice exhibit [redacted] Mouse weight monitoring showed that during the first month of life, [redacted] mice were [redacted] than their [redacted] littermates ([redacted]). We might be forced to start [redacted] experiments by pulling four [redacted] mice. For 25 [redacted] experiments on [redacted] mice, we would need 100 (25X4) [redacted] mice. 1000 [redacted] mice are used for breeding, 25% is [redacted] and ~ 40% survives, generating 100 [redacted] mice.

To be able to perform [redacted] experiments on [redacted] mice, 400 animals are bred, 25% is [redacted] (n = 100).

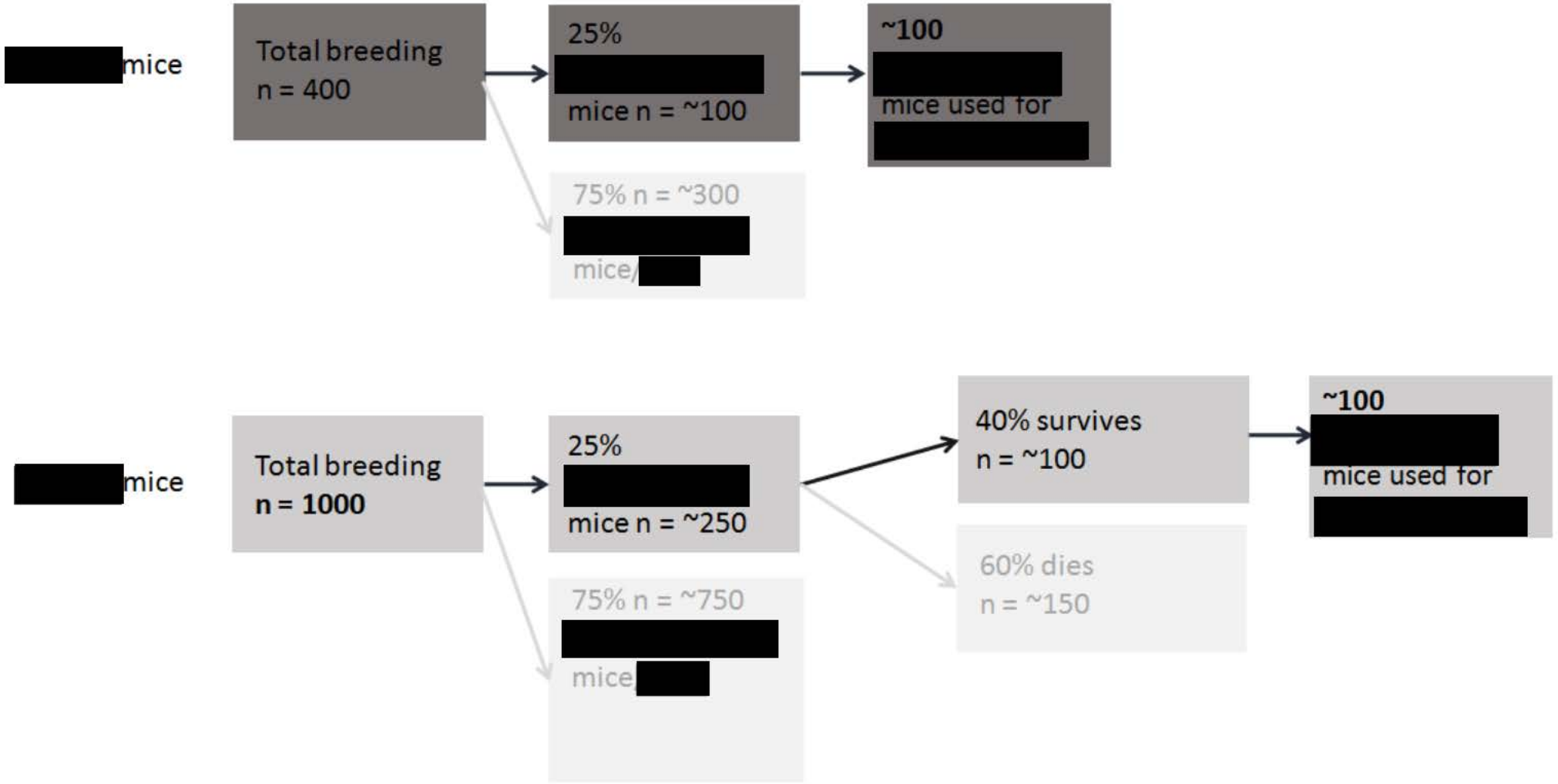


Figure 1: [redacted] and [redacted] mice included in [redacted] experiments.

Species	Origin	Maximum number of animals	Life stage
[redacted]	[redacted] line	100	From > [redacted]
	line	250	From >

C. Re-use

Will the animals be re-used?

[X] No, continue with question D.
[] Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

[] No
[] Yes > Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Keeping the [redacted] line will only be done with [redacted] breeding, eliminating the birth of [redacted] mice with [redacted] [redacted] Only when experiments are planned breeding with [redacted] will start, resulting in 25% [redacted] mice. Mice will be identified via genotyping as soon as possible. [redacted] and [redacted] littermates are used to set up the protocol and optimize the [redacted] procedure. No extra mice are bred for these purposes. The DM1 model is carefully considered. We build on expertise gathered from published data and experiments performed previously in our own research group and by collaborators. By optimizing the process of [redacted], [redacted] and amplification we aim to generate the maximum number of [redacted] usable as [redacted] from one [redacted]. Thereby we minimize the number of mice needed for [redacted] experiments.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

All measures are taken to minimise suffering, pain, fear and other adverse effects. Disturbances in welfare are kept to a minimum. To detect unexpected discomfort in an early stage animals are watched closely and will be weighed regularly. Especially the [redacted] mice are monitored carefully on a daily basis during the [redacted]. It is important to monitor weight and food intake. If [redacted] mice

start to develop [redacted], however this is stressful for the animals. When mice will only be in experiments for another foreseeable/short time period, they are fed water soaked soft food. This to allow the mice to eat while preventing the stressful procedure of teeth clipping. Mice do not experience fear, pain or suffering related to the [redacted].

Repetition and Duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

The proposed study has not been performed before. [redacted] of [redacted] and [redacted] from mouse [redacted] has been performed previously on [redacted] mice and on [redacted] tissue of [redacted] mice. However [redacted] of [redacted] has never been performed on DM1 mice models. We are the first group that started a pilot on two [redacted] mice showing that [redacted] is possible from this [redacted] tissue. Online databases show no articles on [redacted] from DM1 patients/ animal models. In addition, no groups in the [redacted] field are working on this. [redacted] the [redacted] cells by [redacted] and applying them as [redacted] against the [redacted] phenotype in DM1 is an innovative promising new research never attempted before.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

[X] No
[] Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

[X] No > Continue with question H.
[] Yes > Describe this establishment.

G. Location where the animals procedures are performed

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

[X] No > Continue with question I.
[] Yes > Will anaesthesia, analgesiaor other pain relieving methods be used?

[] No > Justify why pain relieving methods will not be used.

[] Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals welfare may be expected?

The DM1 mice have an [redacted]. This genetic alteration leads to the [redacted] in [redacted] mice. [redacted] exhibit [redacted]. Mouse weight monitoring showed that during the first month of life, [redacted] mice were much [redacted] than their [redacted] littermates (about 50%). After 2 months of age, [redacted] females and males caught up in [redacted] (70–80% for females, and 60–70% for males) ([redacted]). There is a [redacted] before weaning for [redacted] mice. We estimated the [redacted]. Mice might start to develop long [redacted]

It is very important to realize that only [redacted] mice can show [redacted] animals with the DM1 [redacted] are bred before. From previous experience we know that DM1 mice show normal cage behaviour in standard environment. Only when challenged differences appear between [redacted] mice and wild-type littermates. No [redacted] pain or discomfort has been reported in literature. The [redacted] mice can only display the DM1 related phenotype after the age of [redacted] and when in a challenging environment. However, most mice are sacrificed at [redacted] and all animals are sacrificed at [redacted]. We can exclude any [redacted] pain, discomfort or changes in cage behaviour to be present.

Explain why these effects may emerge.

Cause of the adverse effects is the [redacted].

Indicate which measures will be adopted to prevent occurrence or minimise severity.

If mice start to develop [redacted], however this is stressful for the animals. When mice will only be in experiments for another foreseeable/short time period, they will be fed water soaked soft food. This to allow the mice to eat while preventing the stressful procedure of teeth clipping. The [redacted] mice experience no discomfort. They show normal cage behavior, no growth retardation and [redacted] or other abnormalities.

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

[X] No > Continue with question K.
[] Yes > Describe the criteria that will be used to identify the humane endpoints.

Indicate the likely incidence.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned (non-recovery, mild, moderate, severe).

Breeding of [redacted] will result in [redacted], [redacted] and [redacted] total 100%). The breeding of [redacted] mice is classified before as moderate in [redacted]. From the [redacted] mice born, [redacted] dies. [redacted] of the [redacted], [redacted] mice that live, are expected to develop [redacted]). Clipping of [redacted] in [redacted] mice is classified as mild. The animals experience short-term mild distress. The cumulative discomfort for [redacted] mice is moderate.
[redacted] mice do not show neonatal death, growth retardation, muscle pain or elephant teeth.
All mice (100%) included in this DAP are sacrificed for [redacted]. There are no treatments, mice are directly sacrificed. This is mild discomfort.
Summary cumulative discomfort:
- 100 [redacted] mice in experiment; mild discomfort (28,5% of the mice).
- 250 [redacted]:
  150 [redacted] mice neonatal death; moderate discomfort (43% of the mice).
  100 [redacted] mice in experiment; moderate discomfort (28,5% of the mice).
Total 350 [redacted] mice (100% of all the mice).

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

[] No > Continue with Section 3: 'Signatures'.
[X] Yes > Explain why it is necessary to kill the animals during or after the procedures.

Animals are sacrificed since we need to [redacted] from dissected [redacted]

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

[] No > Descripe the method of killing that will be used and provide justifications for this choice.

[X] Yes

5

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website www.zbo-ccd.nl.
- Or contact us by phone. (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10300	
1.2	Provide the name of the licenced establishment.	Stichting Katholieke Universiteit Nijmegen	
1.3	List the different types of animal procedures. Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.	Serial number 2	Type of animal procedure 3.4.4.2 [redacted] application of [redacted] or saline solution

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

With this protocol we aim to identify both functional and histological effects of [redacted] with [redacted] and [redacted]. By implementing both [redacted] and [redacted] we are able to investigate the therapeutical value. Two different questions are answered by the approaches:

1. [redacted] will enable us to [redacted] model. This will provide information about [redacted]
2. [redacted] allows us to study the [redacted] This will provide information about [redacted].

The [redacted] have an inhibited immune system. Consequently, these mice can receive cells [redacted] tissues without presenting a rejection response. This will provide information about feasablility of the [redacted]. The [redacted] will be bought. [redacted] of enough [redacted] from [redacted] biopsy material will be a challenge. With the [redacted] technique an unlimited number of [redacted] can be generated from [redacted] cells of DM1 patients. [redacted] of [redacted] derived [redacted] into [redacted] can then show the potential of human [redacted].

For all approaches, we will determine the contribution of [redacted] to [redacted] For immunohistochemistry, [redacted] will be collected and stained using antibodies against [redacted] proteins such as dystrophin, laminin and myosin heavy chain. In vitro analysis of [redacted] function in a tissue bath is done as described by Park et al (J. Vis Exp. 2012). Functional measurements will be performed to compare motor capacity (e.g. treadmill running) and muscle [redacted] in [redacted] and [redacted] muscle as previously done for [redacted] research.

In the end, we expect to reduce [redacted]-related [redacted] symptoms by [redacted] and witness: [redacted], [redacted] strength, reduced [redacted] and increased [redacted] [redacted]

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

We want to investigate the ability of [redacted] to contribute to functional [redacted] To assess the *in vivo* [redacted] we will inject [redacted] in the [redacted] mice. Functional measurements to compare [redacted] will include an [redacted] on a [redacted] The mice will be allowed to settle for 2 minutes with the treadmill belt stationary. After acclimatization with gentle walking for two minutes (speed of 4m/min) and a warm up (8m/min) the main exercise session starts (30 min at 12m/min). [redacted] measurements can be performed by placing the mice on a grid while gently pulling their tails in the opposite direction. The maximal strength exerted by the mouse before releasing should be recorded multiple times with a short recovery in between. The mean of the recorded measurements is the [redacted]. Mice will be tested before (baseline measurement) and several times after cell treatment. With a maximum of twice a week. A decrease in [redacted] strength can be measured around 10 months of age therefor animals are kept up to a maximum of 12 months after [redacted] As a starting point we will analyze [redacted] after [redacted]. After the last functional test mice will be sacrificed and [redacted] will be collected for immunohistochemistry. The numbers of [redacted] that have successfully [redacted] are counted. In this manner, we can analyze the contribution of [redacted].
Based on the outcome of these initial experiments we will vary the [redacted] We aim to start the [redacted] with 5 x 10^5 [redacted] with a maximum of 20 [redacted] (max 1x10^7 [redacted] total). One [redacted] will induce temporary mild stress and a short-term painful sensation of the [redacted]

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

We will gather experience from the group of [redacted] and the group of [redacted]. The first group is working in the DM1 field and experienced with [redacted] while the latter group set up the [redacted] protocol and is working with other [redacted] animal models. They can provide the first guidelines on [redacted], time points and doses. [redacted] treatment is given to one limb, with the other limb [redacted] serving as a control and receiving saline solution. This approach provides us with a valid control for treatment group and minimizes the number of animals needed.

Optimization in the form of pilot experiments (n=3) will still be necessary. After this, reliable statistics for pricise group size can be performed. The first trials and following optimization will be done with de [redacted] mice. These mice are easier to breed and experience less discomfort. Therefore the number of [redacted] mice included in [redacted] is higher (n=50) than the number of [redacted] mice included in DAP3 (n=36). [redacted] mice (n=12) are included to study the behaviour of human cells in vivo. These mice are bought.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

As mentioned before we aim to identify both functional and histological effects of [redacted] with control and [redacted]

[redacted]. By implementing both [redacted] cells we are able to investigate the total therapeutical value. Two different questions are answered by the approaches:

1. [redacted] will enable us to [redacted] model. This will provide information about [redacted].
2. [redacted] allows us to study the [redacted]. This will provide information about [redacted].

The [redacted] have an inhibited immune response. Therefor we can [redacted] without a rejection response. This will provide information about feasibility of the [redacted] Whilst [redacted] has ample expierence with breeding of DM1 mice, the 12 [redacted] mice will be bought.

It is important to consider that only [redacted] mice are able to express a [redacted] when in a challenging environment. These [redacted] DM1 mice are important models to investigate the functional effects of [redacted]. In addition, the protocol deals with many variables such as [redacted] doses, [redacted] sites etc. For [redacted] the variables time points, [redacted] number and location need to be considered. For these three variables our experiences teaches us that we need 12 animals per group (n=36). We will start with a small pilot study (n=3) with [redacted] into [redacted] old mice. The optimization will be done with de [redacted] mice. These mice are easier to breed and experience less discomfort. Therefore the number of [redacted] mice included in [redacted] is higher (n=50) than the number of [redacted] mice included (n=36). We will pay attention to cell survival and contribution to [redacted] Depending on the outcome we will change [redacted] time, dosis and frequency.

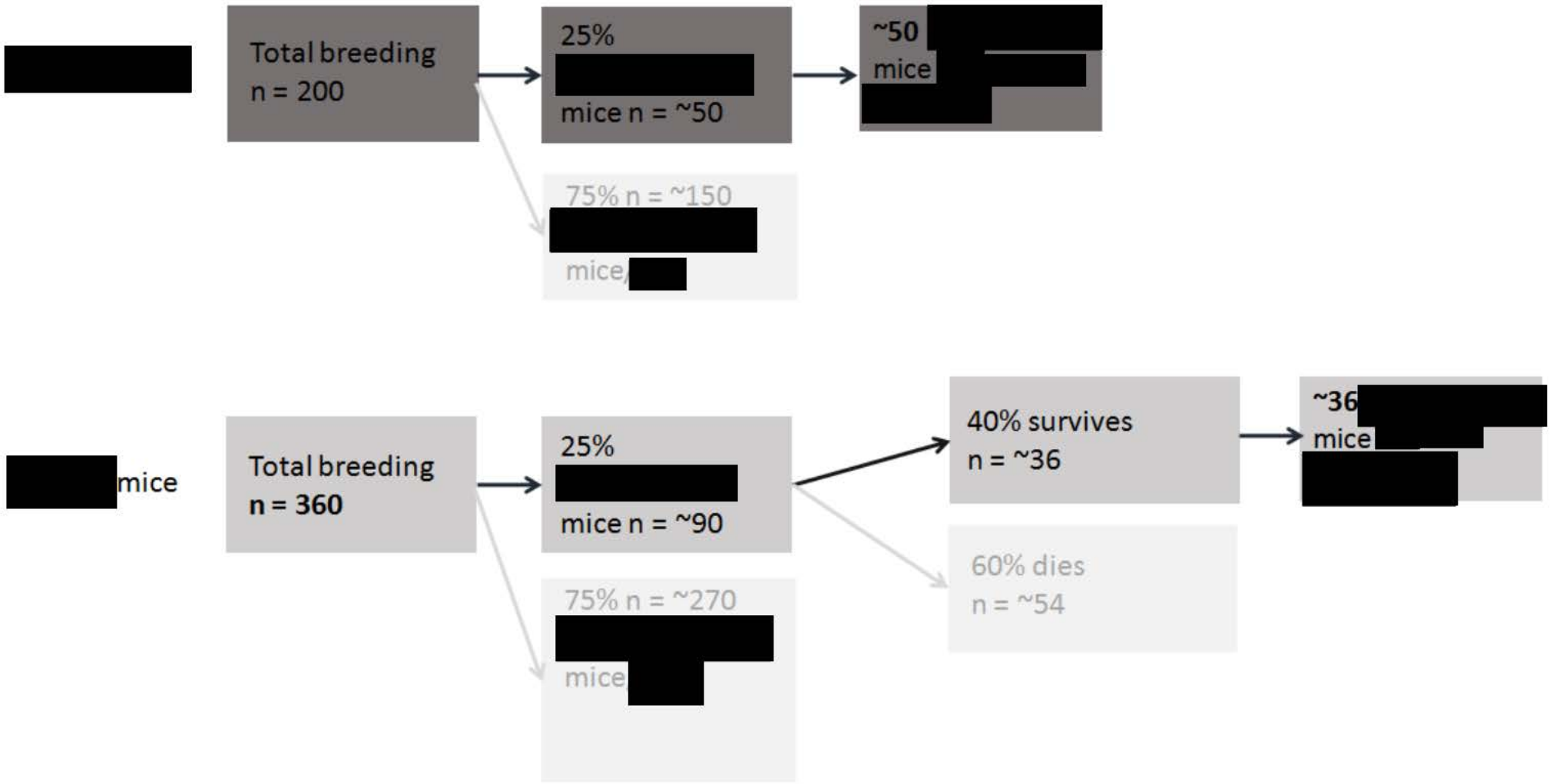


Figure 2: [redacted] into [redacted] and [redacted] mice.

Species	Origin	Maximum number of animals	Life stage
[redacted]	[redacted]	50 90 12	[redacted]

C. Re-use

Will the animals be re-used?

[X] No, continue with question D.
[] Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

[] No
[] Yes > Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

[redacted] mice of the [redacted] line are the only suitable mice to test the [redacted]. The [redacted] mouse model is the only [redacted] model with a functional phenotype in a challenging environment. Testing the effect of [redacted] can only be done in [redacted] mice. To optimally design the protocol we aim to use as few animals as possible while still obtaining as statistically valid results. We build on expertise gathered from other groups [redacted]), published data and experiments performed previously in our own research group. [redacted] [redacted] treatment is given to one limb, with the other limb muscle serving as a control and receiving saline solution. This approach minimizes the number of mice needed.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

We aim for the most optimal treatment as mimimal invasive as possible. Meaning that we aim to reduce the numbers of [redacted] to an absolute minimum to reduce animal suffering.

Repetition and Duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

Administration of muscle [redacted] is a up-and-coming approach for the treatment of [redacted]. In models of [redacted] of [redacted] is being explored ([redacted] However, research only just started. Our approach against DM1 is even more promising as it is [redacted]l and applicable to a broad patient spectrum. Online databases show no articles on [redacted] from DM1 patients/models. [redacted] the [redacted] and applying them as [redacted] against the [redacted] phenotype in DM1 is innovative promising new research never attempted before.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

[X] No
[] Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

[X] No > Continue with question H.
[] Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

[] No > Continue with question I.
[X] Yes > Will anaesthesia, analgesiaor other pain relieving methods be used?

[X] No > Justify why pain relieving methods will not be used.

No anaesthesia, analgesia or other pain releving methods are used. Pain experienced is due to the [redacted] ([redacted]). Only short term mild pain and distress are induced. There is no use in giving pain relief as this will be an injection itself.

[] Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals welfare may be expected?

[redacted] mice will display an [redacted] phenotype in a challenging evironment. From previous experience we know that [redacted] mice show normal cage behaviour in standard environment. Only when challenged differences appear between [redacted] mice and wild-type littermates. No muscle pain or discomfort has been reported in literature. We expect a decrease in [redacted] from 4 months of age and decrease in [redacted] around 10 months of age. [redacted] exhibit [redacted] Mouse weight monitoring showed that during the [redacted], [redacted] mice were smaller than their [redacted] littermates (about 50%). After 2 months of age, [redacted] females and males [redacted] in [redacted] (70–80% for females, and 60–70% for males) [redacted]. Mice might start to develop [redacted] [redacted] (in [redacted] and [redacted] with [redacted] are administered with a starting point of 5 x 10^5 [redacted] with a maximum of 20 [redacted] (max 1x10^7 [redacted] total). One [redacted] will induce temporary mild stress and a short-term painful sensation of the [redacted]

Explain why these effects may emerge.

Cause of the adverse effects are 1) the [redacted], 2) the [redacted].

Indicate which measures will be adopted to prevent occurrence or minimise severity.

We aim to reduce the numbers of [redacted] to an absolute minimum to reduce animal suffering. Possible infections or wounds as a consequence of [redacted] will be monitored closely. We do not expect to see any adverse events as a consequence of [redacted]
If mice start to develop [redacted], however this is stressful for the animals. When mice will only be in experiments for another foreseeable/short time period, they are fed water soaked soft food. This to allow the mice to eat while preventing the stressful procedure of [redacted].

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

[] No > Continue with question K.
[X] Yes > Describe the criteria that will be used to identify the humane endpoints.

We will euthanize a mouse if any of the following signs of discomfort arise between [redacted] and programmed sacrifice and the symptoms do not diminish/disappear in a few days:

- Self-mutilation: excessive licking of the area, biting, scratching.
- Isolation: stays in the corner of the cage, does not interact with cage mates.
- Change in posture: hunching, huddling, stiff movement, head down.

Indicate the likely incidence.

We expect the incidence of humane endpoints to be zero or very low.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned (non-recovery, mild, moderate, severe).

Immunodeficient, [redacted] and [redacted] mice are included in this DAP. All mice (100%) included will experience short term mild pain as a consequence of [redacted]. We expect no complications as the [redacted] fluid is composed of sterile [redacted] cells. The functional measurements

performed on all mice (100%) included can be slightly stressful the first time around however, they will not cause pain and are therefore classified as mild discomfort. The [redacted] and [redacted] mice do not develop [redacted]. Breeding of [redacted] mice is classified as moderate and the [redacted] mice develop [redacted] We expect this to occur in ~45% of [redacted] mice. The discomfort is mild.
Summary cumulative discomfort:
- 50 [redacted] + 12 [redacted] in experiment; 62 mice, mild discomfort (40% of the mice).
- 90 [redacted]:
  54 [redacted] mice [redacted]; moderate discomfort (36 % of the mice).
  36 [redacted] mice in experiment; moderate discomfort (24 % op the mice).
Total 152 mice (100% of all mice).

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

[] No > Continue with Section 3: 'Signatures'.
[X] Yes > Explain why it is necessary to kill the animals during or after the procedures.

Animals are sacrificed. We aim to investigate the therapeutic potential of [redacted]. To analyze the histological effects of the [redacted] we need to examine skeletal muscles.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

[] No > Descripe the method of killing that will be used and provide justifications for this choice.

[X] Yes

6

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website www.zbo-ccd.nl.
- Or contact us by phone. (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10300	
1.2	Provide the name of the licenced establishment.	Stichting Katholieke Universiteit Nijmegen	
1.3	List the different types of animal procedures. Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.	Serial number 3	Type of animal procedure 3.4.4.3 [redacted] application of [redacted]

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

If we succeed to obtain positive results with [redacted] of [redacted], our next aim is to assess the functional incorporation of the [redacted] in [redacted] tissue after [redacted] application. This is a very important research step, since we intent to translate the therapeutic approach to the clinic. After [redacted].

[redacted] administration will be applied to the [redacted] mice. The [redacted] mice show progressive [redacted] from 4 months of age. This is due to [redacted] present at 3 months of age. Mild phenotypic [redacted] is also observed in 4 to 18 months old [redacted] mice ([redacted]). As suggested by [redacted] et al. we will set up a therapeutic pilot trial with [redacted] old [redacted] mice ([redacted]). The [redacted] old [redacted] remains constant between [redacted] of age, improvements not disease stabilization by a therapy could therefor be demonstrated. [redacted] are the only [redacted] that will be [redacted]. These are the only [redacted]; thereby allowing [redacted] delivery towards the [redacted]. These properties have also prompted investigations towards the use of [redacted] in the treatment [redacted]). Moreover, very recently a phase I/II clinical study started treatment with [redacted] transplantation of donor [redacted] in human proved to be feasible and safe ([redacted]

To minimize the use of animals and obtain the most effective approach we will start with a small pilot study of 3 mice. We expect [redacted] to home to [redacted], not to other tissues. To enhance this principle mice are subjected to [redacted] of the [redacted]. This is classified as short term moderate impairment of general well being.
Mice are anesthetized, shaved and disinfected. An incision in the inguinal region is performed, the [redacted], [redacted] are [redacted] into the [redacted]. The wound is then disinfected, closed with sutures and antibiotics and analgesics are administered. This approach has already succesfully been applied in a mouse model of [redacted] The technique is applied in the lab of Prof. Sampaolesi were I am currently learning the procedures needed to start this project.
[redacted] is preferred over [redacted] as the first is the only efficient way of [redacted]. [redacted] of [redacted] into the [redacted] showed that 30 ± 7% of the [redacted] were detected in the [redacted] of the [redacted]. Only <3% of [redacted] had occurred through the [redacted]. [redacted] delivered [redacted] towards all downstream [redacted], especially in areas where degeneration and regeneration was occurring ([redacted]

We will analyze the effect of [redacted] treatment in [redacted] mice with functional measurements. There is mild impairment in general well being due to the training for functional motor measurements comparing [redacted], muscle force etc. These parameters can be assessed by a [redacted] test, time-to-exhaustion assay [redacted]) and measurement of [redacted] properties. Of course, histological incorporation of [redacted] is investigated. When [redacted] home to [redacted] and contribute to [redacted] we will plan a follow up study adjusting and further optimizing the [redacted] application (eg [redacted] time point, [redacted] dosis).

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

First, mice are trained for functional [redacted] measurements. [redacted] function in mice can be assessed by measuring locomotion, strength, balance/coordination, and endurance capacity. For example, mice [redacted] strength can be evaluated on a weekly basis by a [redacted] test. Mice are placed on a [redacted] and gently [redacted] in the opposite direction. The maximal strength exerted by the mouse before releasing their grip is recorded. The mean of around five measurements is taken as the index of [redacted] strength [redacted] et al., Neuromuscul Disord., 2010).
Secondly, the animals are exposed to [redacted]. A single [redacted] will be applied a[redacted] prior to [redacted] to [redacted], a [redacted] for [redacted]. We expect to measure a >2 fold increase in IL, integrins and NF-kB in a small blood sample taken from the tail vein. Efficient engraftment of bone marrow derived stem cells (BMDC's) has previously been accomplished by forced [redacted]. Mice remained at basal conditions or [redacted] This will be our starting point [redacted]).To gain experience in functional measurements we will seek collaboration with other research groups in the [redacted] field already using functional read-outs (e.g. [redacted]).
Finally, [redacted] are [redacted] into the [redacted] mice. As suggested by [redacted] et al. we will use [redacted] old [redacted] mice ([redacted]. The [redacted] old [redacted] [redacted] remains constant between [redacted], improvements not disease stabilization by a therapy could therefor be demonstrated. A control group receiving placebo (saline solution) is included. Functional measurements are performed until a few weeks after [redacted] Mice are sacrificed and tissues analysed. We expect histological analysis to show homing of [redacted] into [redacted] tissue and involvement of [redacted].

The absence of adverse effects and contribution of [redacted] to [redacted] will allow the design of a larger optimized follow-up trial.

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

We will investigate the feasability of the [redacted] approach before starting a follow up study to prove the effectiveness of the treatment. It is important to consider that only [redacted] mice ([redacted] of the pups born) exhibit a [redacted] phenotype, of which only [redacted] survives. Only these [redacted] mice are used to investigate the [redacted]. In addition, this protocol deals with many variables such as [redacted] time points, injection dosis, [redacted] sites etc. We will gather experience from the group of [redacted] and the group of [redacted]. The first group is working in the [redacted] field and experienced with the mouse model while the latter group set up the [redacted]

[redacted] protocol and is working with other [redacted] animal models. Optimization in the form of small pilot experiments (n=3) will still be nessecary. Most animals will be used to test [redacted] application. Only a few mice will undergo [redacted] application of the [redacted]. The exact numbers depend on the outcome of [redacted] administration (protocol 3.4.4.2). We aim for two trials of n=7 (total n=14).

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

We will start with a feasability study injecting three [redacted]-old [redacted] [redacted] mice with [redacted]). When [redacted] home to [redacted] and [redacted] 4 and 12 months of age, improvements not disease stabilization by a therapy could therefor be demonstrated. After proving the feasability we can optimize the effectiveness of the treatment.

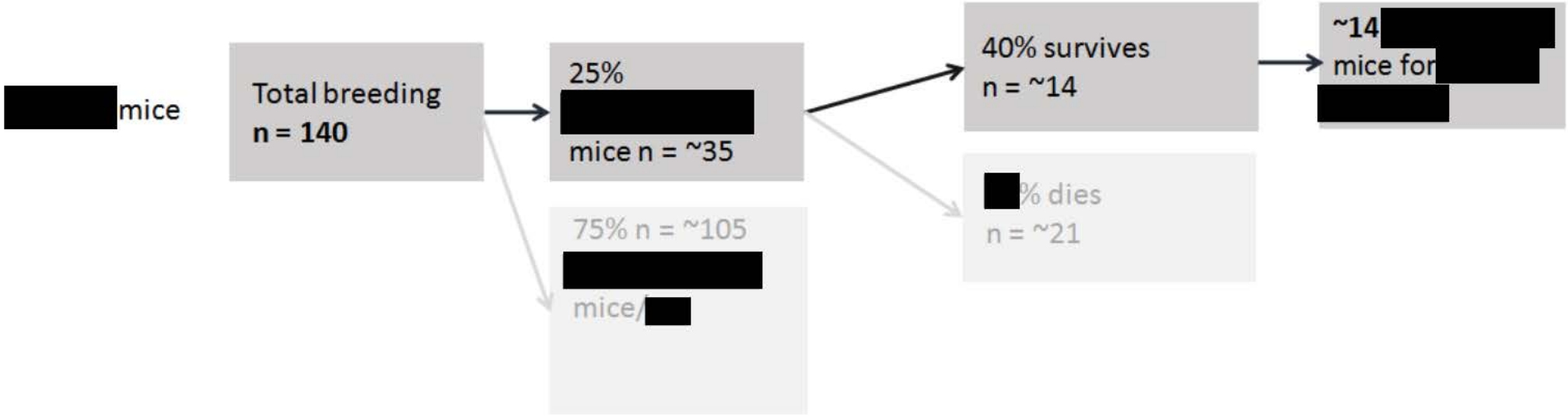


Figure 3: [redacted] into [redacted] mice.

Species	Origin	Maximum number of animals	Life stage
[redacted]	[redacted] line	35	Mature adults ~ [redacted]

C. Re-use

C. Re-use

Will the animals be re-used?

[X] No, continue with question D.

[] Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

[] No

[] Yes > Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

To study the effectiveness of [redacted], the [redacted] mouse is a good fit; it shows [redacted] and [redacted] important disease characteristics seen in patients. To optimally design the protocol we aim to use as few animals as possible while still obtaining statistically valid results. By performing a small pilot study we are able to further refine and reduce animal experiments. We build on expertise gathered from collaborations, published data, statistics and experiments performed previously in our own research group.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

Animals will be watched closely and will be weighed regularly consequently, discomfort can be established in an early stage. Moreover, humane endpoints are established to minimize suffering.

Repetition and Duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

E. Repetition

The proposed study has not been performed before. The [redacted] is a leading institute in the field of [redacted] research. Investigations to the role of [redacted] in [redacted] are promising and widely studied, however the potential of these cells has never been studied with regards to [redacted] This is confirmed by a literature search.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

[X] No
[] Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

[X] No > Continue with question H.
[] Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

H. Pain and pain relief

[] No > Continue with question I.

[X] Yes > Will anaesthesia, analgesiaor other pain relieving methods be used?

[] No > Justify why pain relieving methods will not be used.

[X] Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Mice are anesthetized with an intra-peritoneal injection. An incision in the inguinal region is performed and the [redacted] is [redacted] for [redacted] The wound is then disinfected, closed with sutures and antibiotics and analgesics are administered. This approach has already succesfully been applied in a mouse model of [redacted], the [redacted], and the [redacted]; [redacted], [redacted] Moreover, on all [redacted] treated animals transcardial perfusion is applied to a.) investigate the contribution of cells to myogenesis and b.) explore [redacted] of [redacted] cells to other tissues. In this case, animals will be euthanized by the fixative under anesthesia.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals welfare may be expected?

From previous experience and literature we know that DM1 mice show normal cage behaviour in standard environment. Only when challenged differences appear between [redacted] mice and wild-type littermates. [redacted] mice might start to develop [redacted] [redacted] mice are exposed to [redacted]. To measure increase in inflammation, a small blood sample is taken from the tail vein before and after [redacted]. Moreover, locomotion is measured by investigating strength, balance/coordination, and endurance capacity which cause mild impairements in general well being.

Explain why these effects may emerge.

Cause of the adverse effects are 1) the [redacted], 2) the [redacted] and 3) the [redacted].

Indicate which measures will be adopted to prevent occurrence or minimise severity.

If mice start to develop [redacted], however this is stressful for the animals. When mice will only be in experiments for another foreseeable/short time period, they are fed water soaked soft food. This to allow the mice to eat while preventing the stressful procedure of [redacted] Possible inflammation or infection as a consequence of the [redacted] is monitored carefully. When those unexpected circumstances arise, do not disappear after a few days and visibly affect the mouse, the animal will be sacrifised (see Human endpoints).

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

[] No > Continue with question K.
[X] Yes > Describe the criteria that will be used to identify the humane endpoints.

We will euthanize a mouse if any of the following signs of discomfort continue to be present after [redacted]:

- Self-mutilation: excessive licking of the area, biting, scratching.
- Isolation: stays in the corner of the cage, does not interact with cage mates.
- Change in posture: hunching, huddling, stiff movement, head down.

Indicate the likely incidence.

We expect the incidence of humane endpoints to be zero or very low.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned (non-recovery, mild, moderate, severe).

Breeding of [redacted] mice is classified as moderate. Locomotion in all (100%) [redacted] mice included is measured by investigating strength, balance/coordination, and endurance capacity which will cause mild impairments in general well being. In addition, the DM1 mice are exposed to [redacted] causing mild impairment. A blood sample is taken and cellular treatment is applied by systemic injections causing short-term moderate discomfort. [redacted] is expected to occur in 45% of [redacted] mice. These animals experience mild discomfort. Due to the breeding (moderate discomfort), multiple treatments causing mild discomfort and the [redacted] causing moderate discomfort the cumulative discomfortlevel for all mice included is moderate.
Summary cumulative discomfort:

- [redacted]
  14 [redacted] mice in experiment; moderate discomfort (40% of the mice).
  21 [redacted] mice [redacted] moderate discomfort (60% of the mice).

Total 35 mice (100% of all the mice).

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

[] No > Continue with Section 3: 'Signatures'.
[X] Yes > Explain why it is necessary to kill the animals during or after the procedures.

Animals are sacrificed. We aim to examine the therapeutic potential of [redacted]. To analyze the histological effects of the [redacted] we need to dissect [redacted].

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

[] No > Descripe the method of killing that will be used and provide justifications for this choice.

[X] Yes

7

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website www.zbo-ccd.nl.
- Or contact us by phone. (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10300	
1.2	Provide the name of the licenced establishment.	Stichting Katholieke Universiteit Nijmegen	
1.3	List the different types of animal procedures. Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.	Serial number 1	Type of animal procedure 3.4.4.1 [redacted] of [redacted] cells from [redacted] [redacted]

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

The overal aim is to investigate whether [redacted]) can contribute to [redacted]. Therefore DM1 mice are bred to 1.) [redacted] and 2.) to administer [redacted] and monitor improvements in [redacted] histology and phenotype.
Mice > [redacted] of age will be sacrificed. We will dissect [redacted] to [redacted] cells. This will be done on the DM1 mouse model, a [redacted] mouse model, and wild-type littermates. The department of [redacted] has experience with breeding of [redacted] and [redacted] mice [redacted]. We need to optimize the process of [redacted] and [redacted] to obtain a.) the most efficient generation protocol ([redacted] of [redacted]), b.) highest percentage of [redacted] with complete [redacted] by [redacted], c.) the maximum number of [redacted] by the maximum amount of amplification/passages. Optimization of these steps will give valuable and useable information about the feasibility to translate the research into clinical practice.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

The DM1 mouse models with [redacted] are from the [redacted] line. To obtain [redacted] mice are sacrificed. Optimization of the procedure is established with [redacted] and [redacted] littermates born from [redacted] breeding. We will use CO2 euthanisation to sacrifise the animals as suggested by the protocol of [redacted]). We can than easily perform [redacted] tissue is cleaned from fat and tendon. Cells of interest are [redacted] characterized and [redacted] is applied for [redacted] This technique has already been applied in our lab for [redacted] from this DM1 mice model. In short, the procedure is as follows: cells are [redacted] with [redacted] and [redacted] by a [redacted]. The [redacted] with a [redacted] allows us to study the [redacted].

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

With respect to the animal procedures; the [redacted] and [redacted] mice will be bred as previously described by our lab in [redacted]. Keeping the [redacted] line will only be done with [redacted] breeding, eliminating the birth of [redacted] mice with [redacted]. Only when experiments are planned breeding with [redacted] will start, resulting in 25% [redacted] mice. Mice will be identified via genotyping as soon as possible. However, it is impossible to identify and euthanize [redacted] pups that will die in the nest. The size of the mice or nest size do not influence chance of survival. The death is [redacted], a time window where we cannot interrupt the nest as this can cause stress and might lead the mother to eat the pups, including the [redacted] pups that do have a survival chance. The cause of [redacted] in the [redacted] [redacted] mice is unknown and never seen in [redacted] or wild-type pups. Children with [redacted] suffer from hypotony and breathing difficulties during birth. Whether this [redacted] is involved in the breeding of [redacted] mice remains undefined. The symptoms reported from adult [redacted] mice resemble the classic [redacted] phenotype instead of [redacted].

We will start [redacted] procedures with small pilot experiments (n=3) based on published/communicated results from other [redacted] models to test the feasibility and further optimize the procedure before starting the preclinical study with statistical significance. By optimizing the process of [redacted] and amplification we aim to generate the maximum number of [redacted] usable as [redacted]) from one [redacted] dissection. Thereby we minimize the number of mice needed for follow up [redacted] experiments. These optimization experiments are performed on 10% of surplus [redacted] mice and wild type littermates born from [redacted] breeding (n=30 for [redacted] and n=75 for [redacted] With these approaches the number of mice is reduced.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

It is established that the [redacted] mouse line is a good animal model for preclinical studies on [redacted] phenotype ([redacted]). Moreover, the model is well known in the [redacted] research area [redacted] and already in house and used in [redacted] studies before at the department of [redacted]. The [redacted] mice show [redacted] No muscle pain or discomfort has been reported in literature. From previous experience we know that DMSXL mice show normal cage behaviour in standard environment. Only when challenged differences appear between [redacted] mice and wild-type littermates. [redacted] [redacted] mice reproduce slight [redacted] and slight [redacted] from 4 to 12 months of age, when in a challenging environment. With the use of the [redacted] model we can perform functional read-outs. [redacted] mice and [redacted] both display an increased, but different [redacted]. The variability in [redacted] in the [redacted] and [redacted] mice is also present in DM1 patients. To most optimally represent the DM1 patient population we want to include both [redacted] and [redacted] mice for [redacted] ánd [redacted] of [redacted] This will provide valuable information as it will confirm that the therapeutic approach is applicable on a broad patient spectrum.

[redacted] mice from [redacted] of age are used for dissection of [redacted] showed that effecient [redacted] can be performed after the first week of birth ([redacted]). Before this age [redacted] of [redacted] is possible. Older mice are expected to yield fewer [redacted] For [redacted] protocols, mice will be kept to a maximum age of [redacted]. After this age, [redacted] of [redacted] becomes difficult (personal communication with [redacted] et al.).
Cells [redacted] from [redacted] are characterized and [redacted]. We prefer including [redacted] models over using control/non-[redacted] mice models since this approach represents the clinical situation. We aim to set up an [redacted] therapy using a patients [redacted] to stimulate [redacted]
We want to perform 25 [redacted] experiments on [redacted] mice. Research on different [redacted] models shows that [redacted] of these [redacted] is most efficient by using [redacted] (pers. communication [redacted] lab). Unfortunately, [redacted] mice exhibit [redacted] Mouse weight monitoring showed that during the first month of life, [redacted] mice were ~50% smaller than their [redacted] littermates [redacted]). We might be forced to start [redacted] experiments by pulling four [redacted] mice. For 25 [redacted] experiments on [redacted] mice, we would need 100 (25X4) [redacted] mice. 1000 [redacted] mice are used for breeding, 25% is [redacted] and ~ 40% survives, generating 100 [redacted] mice.
To be able to perform [redacted] experiments on [redacted] mice, 400 animals are bred, 25% is [redacted] (n = 100).

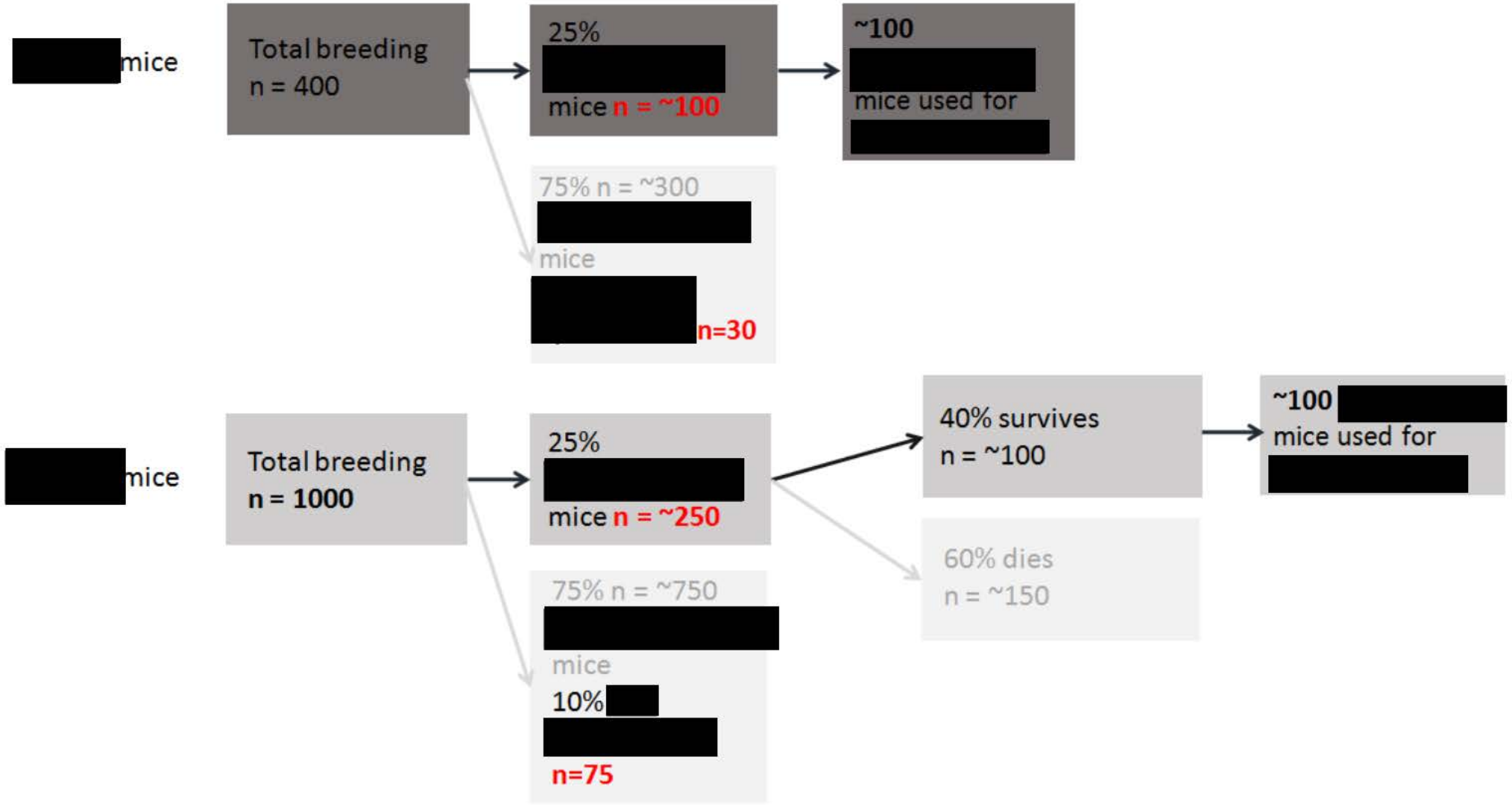


Figure 1: [redacted] and [redacted] mice included in [redacted] experiments.

Species	Origin	Maximum number of animals	Life stage

[redacted]	[redacted] line	130	From > [redacted]
	line	325	From > [redacted]

C. Re-use

Will the animals be re-used?

[X] No, continue with question D.
[] Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

[] No
[] Yes > Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement:

- Our ultimate aim is to treat patients with [redacted] It is therefore important to use [redacted], since these [redacted]. To investigate the potential of this approach we need an animal model in which we can [redacted] in a similar approach. The [redacted] mouse models are the only available models in the world to test the promising [redacted] technique. Therefore, replacement is not an option.

Reduction:

- Keeping the [redacted] line will only be done with [redacted] breeding, eliminating the birth of [redacted] mice with [redacted]. Only when experiments including [redacted] animals are planned breeding with [redacted] will start, resulting in 25% [redacted] mice.
- Both males and females are included in our experiments.
- [redacted] and [redacted] littermates are surplus animals. These mice will be used to set up the protocol and optimize the [redacted] [redacted] procedure. No extra mice will be bred for these purposes.

- The [redacted] model is carefully considered. We build on expertise gathered from published data and experiments performed previously in our own research group and by collaborators. By optimizing the process of [redacted] and amplification we aim to generate the maximum number of [redacted] usable as [redacted]) from one [redacted]. Thereby we minimize the number of mice needed for [redacted] experiments.

Refinement:

- Mice from [redacted] breeding will be identified via genotyping as soon as possible. Especially HOM mice will be closely monitored for abnormal teeth growth.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

All measures are taken to minimize suffering, pain, fear and other adverse effects. Disturbances in welfare are kept to a minimum.
To detect unexpected discomfort in an early stage animals are genotyped as soon as possible and especially [redacted] mice are watched closely and can be weighed regularly. Strict monitoring on a daily basis during the first few weeks after birth is important. During this time period, the number of pups in the nest might differ from day to day since [redacted] mice have a lower change of survival. It is impossible to detect which mice have reduced chances of survival. Therefore we cannot act on possible early death.
After the first few weeks of birth, monitoring is important as [redacted] mice might [redacted]). [redacted] will have to be [redacted], even though this is stressful for the animals. When mice will only be in experiments for another foreseeable/short time period, they are fed water soaked soft food. This is to allow the mice to eat while preventing the stressful procedure of [redacted].Mice do not experience fear, pain or suffering related to the [redacted] because they are dead.

Repetition and Duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

The proposed study has not been performed before. [redacted] from mouse [redacted] has been performed previously on [redacted] mice and on [redacted]) mice. However [redacted] of [redacted] has never been performed on [redacted] mice models. We are the first group that started a pilot on [redacted] mice showing that [redacted] is possible from this [redacted] tissue. Online databases show no articles on [redacted] from [redacted] patients/ animal models. In addition, no groups in the neuromuscular field are working on this. [redacted] the [redacted] [redacted] [redacted] [redacted] by [redacted] and applying them as [redacted] against the neuromuscular phenotype in [redacted] is an innovative promising new research never attempted before.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

[X] No
[] Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

[X] No > Continue with question H.
[] Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

[X] No > Continue with question I.
[] Yes > Will anaesthesia, analgesiaor other pain relieving methods be used?

[] No > Justify why pain relieving methods will not be used.

[] Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals welfare may be expected?

The [redacted] mice have an [redacted]. This genetic alteration leads to the [redacted] phenotype in [redacted] mice. [redacted] exhibit [redacted] Mouse weight monitoring showed that during the first month of life, [redacted] were much smaller than their [redacted] littermates (about 50%). After 2 months of age, [redacted] females and males caught up in weight (70–80% for females, and 60–70% for males) [redacted] There is a high frequency of death before weaning for [redacted] mice. We estimated the mortality about 60% before 1 month of age. Mice might start to develop [redacted]

It is very important to realize that only [redacted] mice can show phenotypical characteristics. [redacted] animals with the [redacted] are bred before. From previous experience we know that [redacted] mice show normal cage behaviour in standard environment. Only when challenged differences appear between [redacted] mice and wild-type littermates. No muscle pain or discomfort has been reported in literature. The [redacted] mice can only display the [redacted] related phenotype after the age of [redacted] and when in a challenging environment. However, most mice are sacrificed at [redacted] and all animals are sacrificed at [redacted]. We can exclude any muscle pain, discomfort or changes in cage behaviour to be present.

Explain why these effects may emerge.

Cause of the adverse effects is the [redacted] in both alelles consisting of the [redacted] in the [redacted]

Indicate which measures will be adopted to prevent occurrence or minimise severity.

If mice start to develop [redacted], however this is stressful for the animals. When mice will only be in experiments for another foreseeable/short time period, they will be fed water soaked soft food. This to allow the mice to eat while preventing the stressful procedure of [redacted]. The [redacted] mice experience no discomfort. They show normal cage behavior, no growth retardation and no elephant teeth or other abnormalities.

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

[X] No > Continue with question K.
[] Yes > Describe the criteria that will be used to identify the humane endpoints.

Indicate the likely incidence.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned (non-recovery, mild, moderate, severe).

Breeding of [redacted] will result in 50% [redacted] mice, 25% wild-type mice and 25% [redacted] mice (total 100%). The breeding of [redacted] mice is classified before as moderate in '[redacted]'. From the 25% of [redacted] mice born, [redacted] dies. 45% of the 40% [redacted] mice that live, are expected to [redacted]). [redacted] mice is classified as mild. The animals experience short-term mild distress. The cumulative discomfort for [redacted] mice is moderate.
[redacted] mice do not show neonatal death, growth retardation, muscle pain or elephant teeth.
All mice (100%) included in this DAP are sacrificed for muscle tissue [redacted] There are no treatments, mice are directly sacrificed. This is mild discomfort.
Summary cumulative discomfort:
- 30 [redacted] mice in experiment; mild discomfort (7% of the mice).
- 100 [redacted] mice in experiment; mild discomfort (22% of the mice).
- 75 [redacted] mice in experment; moderate discomfort (16% of the mice).
- 250 [redacted]:
  150 [redacted] mice neonatal death; moderate discomfort (33% of the mice).
  100 [redacted] mice in experiment; moderate discomfort (22% of the mice).
Total 455 [redacted] mice (100% of all the mice).

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

[] No > Continue with Section 3: 'Signatures'.
[X] Yes > Explain why it is necessary to kill the animals during or after the procedures.

Animals are sacrificed since we need to [redacted] [redacted] from dissected [redacted] tissue.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

[] No > Descripe the method of killing that will be used and provide justifications for this choice.

[X] Yes

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website www.zbo-ccd.nl.
- Or contact us by phone. (0900-2800028).

8

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10300	
1.2	Provide the name of the licenced establishment.	Stichting Katholieke Universiteit Nijmegen	
1.3	List the different types of animal procedures. Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.	Serial number 2	Type of animal procedure 3.4.4.2 [redacted] application of [redacted] [redacted] or saline solution

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

With this protocol we aim to identify both functional and histological effects of [redacted] with control and [redacted]. By implementing both [redacted] we are able to investigate the therapeutical value. Two different questions are answered by the approaches:

1. [redacted] will enable us to [redacted] in a [redacted] This will provide information about [redacted].
2. [redacted] allows us to study the [redacted] This will provide information about [redacted].

The [redacted] mice have an inhibited immune system. Consequently, these mice can receive [redacted] from human tissues without presenting a rejection response. This will provide information about feasablility of the (human) [redacted]. The [redacted] will be bought. [redacted] of [redacted] cells from human biopsy material will be a challenge. With the [redacted] technique an unlimited number of [redacted] can be generated from somatic cells of [redacted] patients. [redacted] of [redacted] derived [redacted] into [redacted] can then show the potential of [redacted].

For all approaches, we will determine the contribution of [redacted]. For immunohistochemistry, [redacted] will be collected and stained using antibodies against [redacted] proteins such as dystrophin, laminin and myosin heavy chain. In vitro analysis of [redacted]) function in a tissue bath is done as described by Park et al (J. Vis Exp. 2012). Functional measurements will be performed to compare motor capacity (e.g. [redacted]) and [redacted]) in [redacted] and [redacted] as previously done for [redacted] research.

In the end, we expect to reduce [redacted]-related [redacted] symptoms by [redacted] and witness: [redacted], [redacted] and [redacted].

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

We want to investigate the ability of [redacted] to contribute to functional [redacted] To assess the *in vivo* [redacted] capacity we will inject [redacted] populations in the [redacted] of mice. Functional measurements to compare [redacted] capacity will include an exercise regimen on a treadmill. The mice will be allowed to settle for 2 minutes with the treadmill belt stationary. After acclimatization with gentle walking for two minutes (speed of 4m/min) and a warm up (8m/min) the main exercise session starts (30 min at 12m/min). [redacted] measurements can be performed by placing the mice on [redacted] while gently pulling [redacted] in the opposite direction. The maximal [redacted] by the mouse before releasing should be recorded multiple times with a short recovery in between. The mean of the recorded measurements is the [redacted]. Mice will be tested before (baseline measurement) and several times after cell treatment. With a maximum of twice a week. A decrease in [redacted] can be measured around 10 months of age therefor animals are kept up to a maximum of 12 months after [redacted] As a starting point we will analyze engraftment, regeneration and functional outcomes [redacted] After the last functional test mice will be sacrificed and [redacted] will be collected for immunohistochemistry. The numbers of [redacted] and control cells that have successfully [redacted] and survived are counted. In this manner, we can analyze the contribution of different cell populations to [redacted]
Based on the outcome of these initial experiments we will vary the transplantation conditions ([redacted] We aim to start the [redacted] with 5 x 10^5 [redacted] with a maximum of 20 [redacted] (max 1x10^7 [redacted] total). One injection will induce temporary mild stress and a short-term painful sensation of the [redacted]

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

We will gather experience from the group of [redacted] and the group of [redacted]. The first group is working in the [redacted] field and experienced with the mouse model while the [redacted] group set up the [redacted] protocol and is working with other [redacted] animal models. They can provide the first guidelines on [redacted] time points and doses. [redacted] treatment is given to one limb, with the other limb muscle serving as a control and receiving saline solution. This approach provides us with a valid control for treatment group and minimizes the number of animals needed.

Optimization in the form of pilot experiments (n=3) will still be necessary. After this, reliable statistics for pricise group size can be performed. The first trials and following optimization will be done with de [redacted] mice. These mice are easier to breed and experience less discomfort. Therefore the number of [redacted] mice included in DAP3 is higher (n=50) than the number of [redacted] mice included in DAP3 (n=36). [redacted] mice (n=12) are included to study the behaviour of human cells in vivo. These mice are bought.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

As mentioned before we aim to identify both functional and histological effects of [redacted] with control and [redacted]

[redacted]. By implementing both [redacted] and [redacted] cells we are able to investigate the total therapeutical value. Two different questions are answered by the approaches:

1. [redacted] will enable us to [redacted] This will provide information about [redacted].
2. [redacted] allows us to study the [redacted]. This will provide information about the possibility to [redacted]

The immunodeficient [redacted] have an inhibited immune response. Therefor we can inject [redacted] without a rejection response. This will provide information about feasibility of the [redacted] Whilst [redacted] has ample expierence with breeding of [redacted] mice, the 12 [redacted] mice will be bought.

It is important to consider that only [redacted] mice are able to express a [redacted] phenotype when in a challenging environment. These [redacted] mice are important models to investigate the functional effects of [redacted]. In addition, the protocol deals with many variables such as [redacted] For [redacted] the variables time points, [redacted] number and location need to be considered. For these three variables our experiences teaches us that we need 12 animals per group (n=36). We will start with a small pilot study (n=3) with [redacted] into [redacted] old mice. The optimization will be done with de [redacted] mice. These mice are easier to breed and experience less discomfort. Therefore the number of [redacted] mice included in DAP3 is higher (n=50) than the number of [redacted] mice included (n=36). We will pay attention to cell survival and contribution to myogenisis. Depending on the outcome we will change [redacted] time, dosis and frequency.

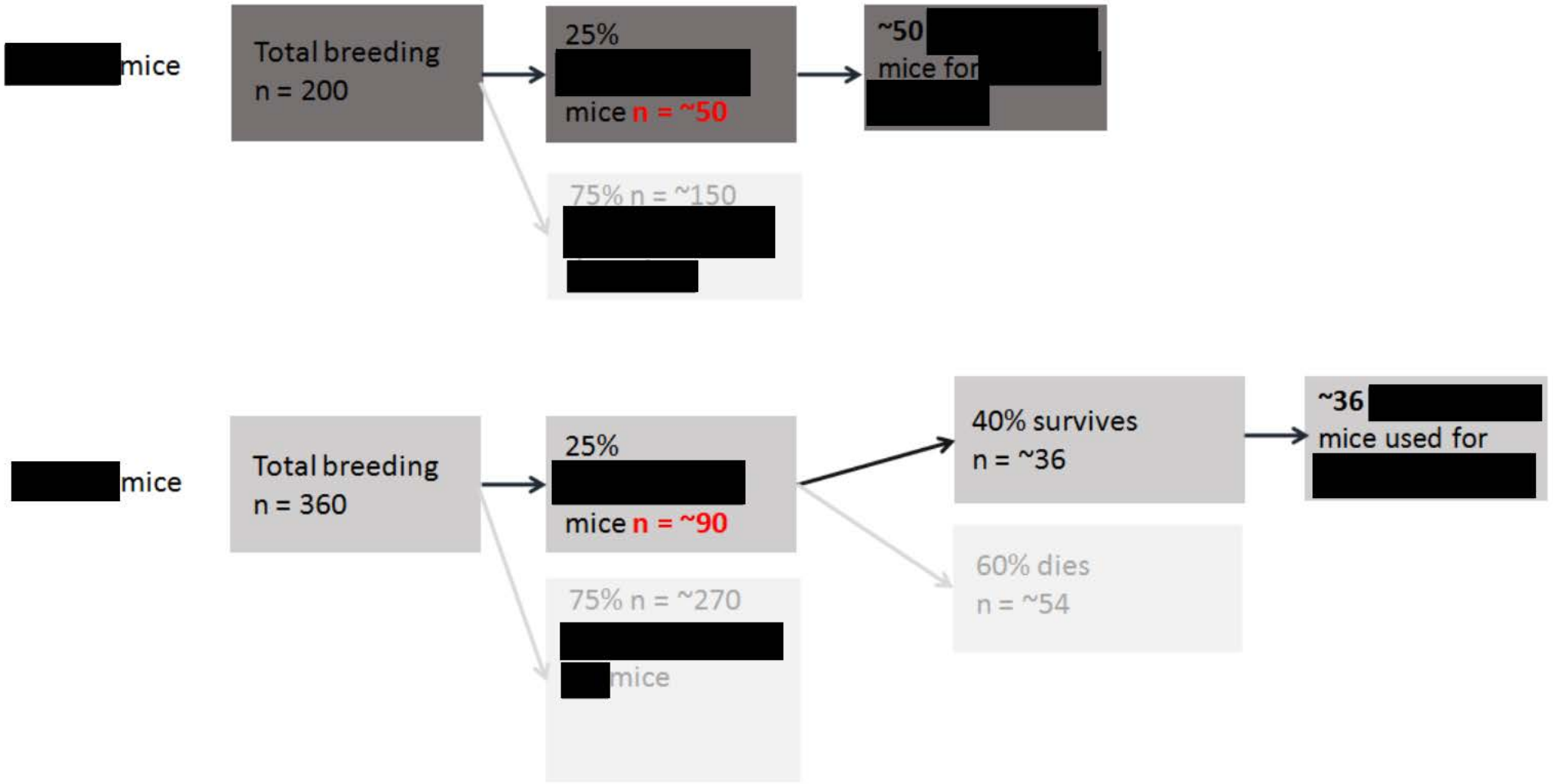


Figure 2: into and mice.

Species	Origin	Maximum number of animals	Life stage
		50 90 12	

C. Re-use

Will the animals be re-used?

[X] No, continue with question D.
[] Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

[] No
[] Yes > Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement:

- We aim to treat patients with [redacted] and [redacted]. It is therefore important to use [redacted], since these [redacted] will not evoke an immune response. To investigate the effectiveness and potential of this approach we need an animal model in which we can a) [redacted] in a similar approach, b) [redacted] and c) [redacted] into the [redacted] and investigate whether they can contribute to [redacted] *in vivo*. We will perform immunohistochemistry and functional measurements such as *in vitro* analysis of [redacted]) function in a tissue bath. For these experiment we need the only mouse models of DM in which we can look at the contribution of [redacted] Consequently, replacement is not an option.
- Testing whether human [redacted] can contribute to [redacted] can only be done in [redacted] mice. The [redacted] mice have an inhibited immune system. Consequently, these mice can receive [redacted] from human tissues without presenting a rejection response. This will provide information about feasibility of the (human) [redacted]. Replacement is not an option.

Reduction:

- To optimally design the protocol we aim to use as few animals as possible while still obtaining statistically valid results. We build on expertise gathered from other groups ([redacted]), published data and experiments performed previously in our own research group. However, optimization in the form of pilot experiments (n=3) is necessary. After the pilot experiment, reliable calculations (in collaboration with a biostatistician) for precise group size can be performed.
- [redacted] treatment is given to one limb, the other limb will serve as a control and will receive saline solution. This approach minimizes the number of mice needed.

Refinement:

- The first trial and following optimization will be done with the [redacted] mice. These mice are easier to breed and experience less discomfort.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

We aim for the most optimal treatment as minimal invasive as possible. Meaning that we aim to reduce the numbers of [redacted] to an absolute minimum to reduce animal suffering, while still holding an effective [redacted]. Outcomes from the pilot experiment will help determining the most optimal but minimal invasive [redacted] protocol.

Repetition and Duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

Administration of [redacted] is a up-and-coming approach for the treatment of muscular dystrophies. In models of [redacted] of [redacted] However, research only just started. Our approach against [redacted] is even more promising as it is [redacted] and applicable to a broad patient spectrum. Online databases show no articles on [redacted] from [redacted] patients/models. [redacted] and applying them as [redacted] against the neuromuscular phenotype in [redacted] is innovative promising new research never attempted before.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

[X] No

[] Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

[X] No > Continue with question H.
[] Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

[] No > Continue with question I.
[X] Yes > Will anaesthesia, analgesiaor other pain relieving methods be used?

[X] No > Justify why pain relieving methods will not be used.

No anaesthesia, analgesia or other pain releving methods are used. Pain experienced is due to the [redacted]). Only short term mild pain and distress are induced. There is no use in giving pain relief as this will be an injection itself.

[] Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals welfare may be expected?

[redacted] mice will display an [redacted] phenotype in a challenging evironment. From previous experience we know that [redacted] mice show normal cage behaviour in standard environment. Only when challenged differences appear between [redacted] mice and wild-type littermates. No muscle pain or discomfort has been reported in literature. We expect a decrease in [redacted] from 4 months of age and decrease in [redacted] around 10 months of age. [redacted] exhibit growth retardation. Mouse weight monitoring showed that during the first month of life, [redacted] [redacted] mice were smaller than their [redacted] littermates (about 50%). After 2 months of age, [redacted] females and males caught up in weight (70–80% for females, and 60–70% for males) [redacted]). Mice might start to develop [redacted] [redacted] (in [redacted] and [redacted] with [redacted] are administered with a starting point of 5 x 10^5 [redacted] with a maximum of 20 [redacted] (max 1x10^7 [redacted] total). One [redacted] will induce temporary mild stress and a short-term painful sensation of the [redacted]

Explain why these effects may emerge.

Cause of the adverse effects are 1) the [redacted], 2) the [redacted].

Indicate which measures will be adopted to prevent occurrence or minimise severity.

We aim to reduce the numbers of [redacted] to an absolute minimum to reduce animal suffering. Possible infections or wounds as a consequence of [redacted] will be monitored closely. We do not expect to see any adverse events as a consequence of [redacted]
If mice start to develop [redacted]), the teeth have to be clipped regular, however this is stressful for the animals. When mice will only be in experiments for another foreseeable/short time period, they are fed water soaked soft food. This to allow the mice to eat while preventing the stressful procedure of [redacted].

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

[] No > Continue with question K.
[X] Yes > Describe the criteria that will be used to identify the humane endpoints.

We will euthanize a mouse if any of the following signs of discomfort arise between [redacted] and programmed sacrifice and the symptoms do not diminish/disappear in a few days:

- Self-mutilation: excessive licking of the area, biting, scratching.
- Isolation: stays in the corner of the cage, does not interact with cage mates.
- Change in posture: hunching, huddling, stiff movement, head down.

Indicate the likely incidence.

We expect the incidence of humane endpoints to be zero or very low.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned (non-recovery, mild, moderate, severe).

[redacted] mice are included in this DAP. All mice (100%) included will experience short term mild pain as a consequence of [redacted]. We expect no complications as the [redacted] fluid is composed of sterile [redacted] The functional measurements performed on all mice (100%) included can be slightly stressful the first time around however, they will not cause pain and are therefore classified as mild discomfort. The [redacted] mice do not develop elephant teeth. Breeding of [redacted] mice is classified as moderate and the [redacted] mice develop [redacted]. We expect this to occur in ~[redacted] of [redacted] mice. The discomfort is mild.
Summary cumulative discomfort:
- 50 [redacted] + 12 [redacted] mice in experiment; 62 mice, mild discomfort (40% of the mice).
- 90 [redacted]:
  54 [redacted] mice [redacted]; moderate discomfort (36 % of the mice).
  36 [redacted] mice in experiment; moderate discomfort (24 % op the mice).

Total 152 mice (100% of all mice).

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

[] No > Continue with Section 3: 'Signatures'.
[X] Yes > Explain why it is necessary to kill the animals during or after the procedures.

Animals are sacrificed. We aim to investigate the therapeutic potential of [redacted]. To analyze the histological effects of the [redacted] we need to examine [redacted].

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

[] No > Descripe the method of killing that will be used and provide justifications for this choice.

[X] Yes

9

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website www.zbo-ccd.nl.
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1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10300	
1.2	Provide the name of the licenced establishment.	Stichting Katholieke Universiteit Nijmegen	
1.3	List the different types of animal procedures. Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.	Serial number 3	Type of animal procedure 3.4.4.3 [redacted] application of [redacted]

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

If we succeed to obtain positive results with [redacted] of [redacted] our next aim is to assess the functional incorporation of the [redacted] in [redacted] tissue after [redacted] application. This is a very important research step, since we intent to translate the therapeutic approach to the clinic. After [redacted] will only reach a [redacted].

[redacted] administration will be applied to the [redacted] mice. The [redacted] mice show progressive [redacted] weakness from 4 months of age. This is due to [redacted] present at 3 months of age. Mild phenotypic [redacted] is also observed in 4 to 18 months old [redacted] mice ([redacted] As suggested by [redacted] et al. we will set up a therapeutic pilot trial with [redacted] old [redacted] mice ([redacted] The [redacted] phenotype remains constant between 4 and 12 months of age, improvements not disease stabilization by a therapy could therefor be demonstrated.
[redacted] are the only [redacted] that will be [redacted] These are the only cells have the capability to [redacted]; thereby allowing [redacted] delivery towards the [redacted] These properties have also prompted investigations towards the use of [redacted] in the treatment [redacted]). Moreover, very recently a phase I/II clinical study started treatment with delivery of [redacted] boys. [redacted] in human proved to be feasible and safe [redacted]

To minimize the use of animals and obtain the most effective approach we will start with a small pilot study of 3 mice. We expect [redacted] to home to [redacted], not to other tissues. To enhance this principle mice are subjected to [redacted] of the [redacted]. This is classified as short term moderate impairment of general well being.
Mice are anesthetized, shaved and disinfected. An incision in the inguinal region is performed, the [redacted] is [redacted] diluted in PBS are [redacted] into the [redacted] The wound is then disinfected, closed with sutures and antibiotics and analgesics are administered. This approach has already succesfully been applied in a mouse model of [redacted] The technique is applied in the lab of [redacted] were I am currently learning the procedures needed to start this project.
[redacted] is preferred over [redacted] as the first is the [redacted]. [redacted] of [redacted] into the [redacted] showed that 30 ± 7% of the [redacted]. Only <3% of [redacted] had occurred through [redacted]. [redacted] delivered [redacted] towards all downstream [redacted], especially in areas where degeneration and regeneration was occurring ([redacted]

We will analyze the effect of [redacted] treatment in [redacted] mice with functional measurements. There is mild impairment in general well being due to the training for functional [redacted] measurements comparing motor capacity, muscle force etc. These parameters can be assessed by a [redacted] test, time-to-exhaustion assay ([redacted]) and measurement of [redacted] properties. Of course, histological incorporation of [redacted] is investigated. When [redacted] [redacted] home to [redacted] [redacted] and contribute to [redacted] we will plan a follow up study adjusting and further optimizing the [redacted] application (eg [redacted] time point, [redacted] dosis).

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

First, mice are trained for functional [redacted] capacity measurements. [redacted] function in mice can be assessed by measuring [redacted] strength, balance/coordination, and [redacted] capacity. For example, mice hindlimb [redacted] can be evaluated on a weekly basis by a grip strength test. Mice are placed on a [redacted] and [redacted] in the opposite direction. The [redacted] exerted by the mouse before releasing their grip is recorded. The mean of around five measurements is taken as the index of hindlimb [redacted] (Vignaud et al., Neuromuscul Disord., 2010).
Secondly, the animals are exposed to [redacted]. We expect to measure a >2 fold increase in IL, integrins and NF-kB in a small blood sample taken from the tail vein. Efficient engraftment of bone marrow derived stem cells (BMDC's) has previously been accomplished by forced [redacted] period. This will be our starting point ([redacted]).To gain experience in functional measurements we will seek collaboration with other research groups in the [redacted] field already using functional read-outs (e.g. [redacted]).
Finally, [redacted] cells are [redacted] into the [redacted] mice. As suggested by [redacted] et al. we will use [redacted] old [redacted] mice ([redacted]). The [redacted] phenotype remains constant between [redacted] of age, improvements not disease stabilization by a therapy could therefor be demonstrated. A control group receiving placebo (saline solution) is included. Functional measurements are performed until a few weeks after [redacted] Mice are sacrificed and tissues analysed. We expect histological analysis to show homing of [redacted] cells into [redacted] tissue and involvement of [redacted] in [redacted]

The absence of adverse effects and contribution of [redacted] to [redacted] will allow the design of a larger optimized follow-up trial.

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

We will investigate the feasability of the [redacted] approach before starting a follow up study to prove the effectiveness of the treatment. It is important to consider that only [redacted] mice ([redacted] of the pups born) exhibit a [redacted] phenotype, of which only [redacted] survives. Only these [redacted] [redacted] mice are used to investigate the [redacted]. In addition, this protocol deals with many variables such as [redacted] time points, [redacted] dosis, injection sites etc. We will gather experience from the group of [redacted] and the group of [redacted]. The first group is working in the [redacted] field and experienced with the mouse model while the latter group set up the [redacted]

[redacted] protocol and is working with other [redacted] animal models. Optimization in the form of small pilot experiments (n=3) will still be nessecary. Most animals will be used to test [redacted] application. Only a few mice will undergo [redacted] application of the [redacted]. The exact numbers depend on the outcome of [redacted] administration (protocol 3.4.4.2). We aim for two trials of n=7 (total n=14).

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

We will start with a feasability study injecting three [redacted]-old [redacted] mice with [redacted]). When [redacted] home to [redacted] and cells seem to contribute to [redacted] a follow up study will start. Since the [redacted] phenotype remains constant between 4 and 12 months of age, improvements not disease stabilization by a therapy could therefor be demonstrated. After proving the feasability we can optimize the effectiveness of the treatment.

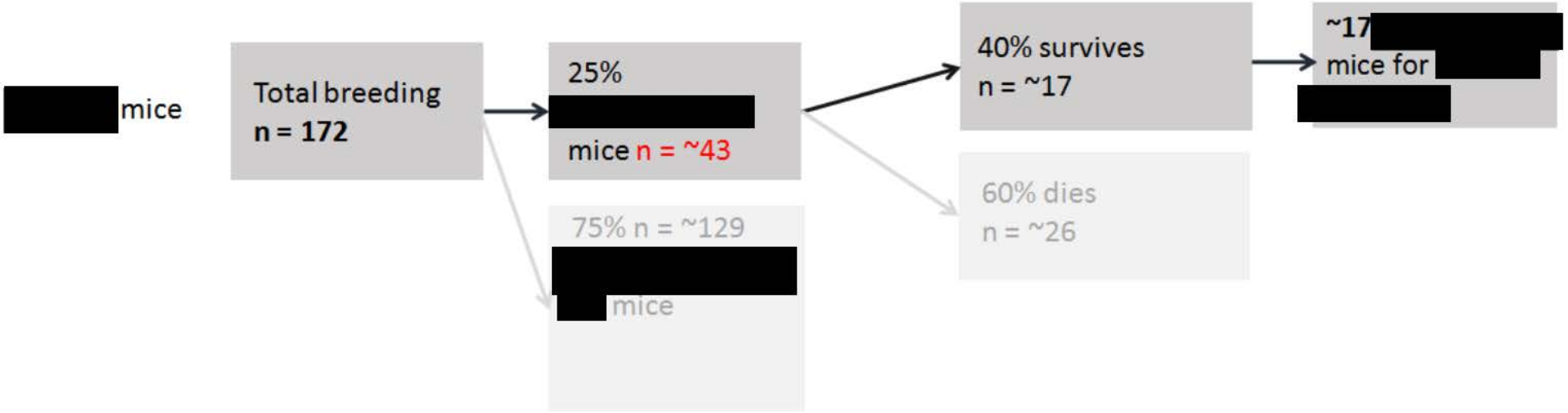

Figure 3: [redacted] into [redacted] mice.

Species	Origin	Maximum number of animals	Life stage
[redacted]	[redacted] line	43	[redacted]

C. Re-use

C. Re-use

Will the animals be re-used?

[X] No, continue with question D.

[] Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

[] No

[] Yes > Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement:

- The [redacted] mouse model shows [redacted] degeneration and [redacted] important disease characteristics seen in patients with [redacted] It is the only [redacted] model with a functional phenotype when in a challenging environment. To study the effectiveness of [redacted], the [redacted] mouse is the only available model.

Reduction:

- Results from [redacted] will be evaluated and a small pilot study will be performed to further reduce the number of mice included in [redacted].

Refinement:

- For [redacted] mice are anesthetized. In addition, antibiotics and analgesics are administered.
- Collaborations are established with groups experienced in functional measurements and [redacted] administration of [redacted]). Techniques and expertise are exchanged to further refine experiments.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

For [redacted], mice are anesthetized and analgesics are administered. Moreover, animals will be closely monitored so that discomfort can be established in an early stage. It is of particular importance to monitor the animals for possible inflammation or infection as a consequence of

the [redacted]. When those unexpected circumstances arise, are visibly affecting the mouse and they do not disappear after a few days, the mouse will be sacrificed. We have established humane endpoints to minimize suffering.

Repetition and Duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

The proposed study has not been performed before. The Radboudumc is a leading institute in the field of [redacted] research. Investigations to the role of [redacted] in [redacted] are promising and widely studied, however the potential of these cells has never been studied with regards to [redacted] This is confirmed by a literature search.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

[X] No
[] Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

[X] No > Continue with question H.
[] Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

[] No > Continue with question I.
[X] Yes > Will anaesthesia, analgesiaor other pain relieving methods be used?

[] No > Justify why pain relieving methods will not be used.

[X] Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Mice are anesthetized with an intra-peritoneal injection. An incision in the inguinal region is performed and the [redacted] is [redacted] for [redacted] The wound is then disinfected, closed with sutures and antibiotics and analgesics are administered. This approach has already succesfully been applied in a mouse model of [redacted] Moreover, on all [redacted] treated animals transcardial perfusion is applied to a.) investigate the contribution of cells to myogenesis and b.) explore [redacted] to other tissues. In this case, animals will be euthanized by the fixative under anesthesia.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals welfare may be expected?

From previous experience and literature we know that [redacted] mice show normal cage behaviour in standard environment. Only when challenged differences appear between [redacted] mice and wild-type littermates. The [redacted] mice might start to develop [redacted] mice are exposed to [redacted]. To measure increase in inflammation, a small blood sample is taken from the tail vein before and after [redacted] Moreover, locomotion is measured by investigating strength, balance/coordination, and endurance capacity which cause mild impairements in general well being.

Explain why these effects may emerge.

Cause of the adverse effects are 1) the [redacted], 2) the [redacted] and 3) the [redacted].

Indicate which measures will be adopted to prevent occurrence or minimise severity.

If mice start to develop [redacted], however this is stressful for the animals. When mice will only be in experiments for another foreseeable/short time period, they are fed water soaked soft food. This to allow the mice to eat while preventing the stressful procedure of [redacted]. Possible inflammation or infection as a consequence of the [redacted] is monitored carefully. When those unexpected circumstances arise, do not disappear after a few days and visibly affect the mouse, the animal will be sacrifised (see Human endpoints).

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

[] No > Continue with question K.
[X] Yes > Describe the criteria that will be used to identify the humane endpoints.

We will euthanize a mouse if any of the following signs of discomfort continue to be present after [redacted]:

- Self-mutilation: excessive licking of the area, biting, scratching.
- Isolation: stays in the corner of the cage, does not interact with cage mates.
- Change in posture: hunching, huddling, stiff movement, head down.

Indicate the likely incidence.

We expect the incidence of humane endpoints to be zero or very low.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned (non-recovery, mild, moderate, severe).

Breeding of [redacted] mice is classified as moderate. Locomotion in all (100%) [redacted] mice included is measured by investigating strength, balance/coordination, and endurance capacity which will cause mild impairments in general well being. In addition, the [redacted] mice are exposed to [redacted] causing mild impairment. A blood sample is taken and [redacted] is applied by [redacted] causing short-term moderate discomfort. [redacted] mice. These animals experience mild discomfort. Due to the breeding (moderate discomfort), multiple treatments causing mild discomfort and the [redacted] causing moderate discomfort the cumulative discomfortlevel for all mice included is moderate.
Summary cumulative discomfort:
- DMSXL HOM:
[redacted] mice in experiment; moderate discomfort (40% of the mice).
mice [redacted]; moderate discomfort (60% of the mice).
Total 43 mice (100% of all the mice).

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

[] No > Continue with Section 3: 'Signatures'.
[X] Yes > Explain why it is necessary to kill the animals during or after the procedures.

Animals are sacrificed. We aim to examine the therapeutic potential of [redacted]. To analyze the histological effects of the [redacted] we need to dissect [redacted].

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

[] No > Descripe the method of killing that will be used and provide justifications for this choice.

[X] Yes

DEC-advies

A. Algemene gegevens over de procedure

1. Aanvraagnummer: 2016-0049
2. Titel van het project: Development of a [redacted] against the [redacted] of Myotonic Dystrophy type 1
3. Titel van de NTS: Ontwikkeling van een celtherapie voor Myotone Dystrofie type 1

4. Type aanvraag:
   ☒ nieuwe aanvraag projectvergunning
   ☐ wijziging van vergunning met nummer

5. Contactgegevens DEC:
   - naam DEC: RUDEC
   - telefoonnummer contactpersoon: [redacted] bereikbaar op maandag, dinsdag, en donderdag van 9:00 tot 15:00 uur
   - e-mailadres contactpersoon: [redacted]

6. Adviestraject (data dd-mm-jjjj):
   ☒ ontvangen door DEC: 22-08-2016
   ☐ aanvraag compleet
   ☒ in vergadering besproken: 06-09-2016 en 08-11-2016
   ☐ anderszins behandeld
   ☒ termijnonderbreking(en) van 13-09-2016 tot 17-10-2016 en van 14-11-2016 tot 08-12-2016
   ☐ besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen
   ☒ aanpassing aanvraag: 17-10-2016 en 16-11-2016
   ☒ advies aan CCD: 21-12-2016

7. De inhoud van dit project is afgestemd met de IvD en deze heeft geen bezwaren tegen de uitvoering van het project binnen deze instelling.

8. Eventueel horen van aanvrager:
   - Datum
   - Plaats
   - Aantal aanwezige DEC-leden
   - Aanwezige (namens) aanvrager
   - Gestelde vraag / vragen
   - Verstrekt(e) antwoord(en)
   - Het horen van de aanvrager heeft wel/niet geleid tot aanpassing van de aanvraag.

9. Correspondentie met de aanvrager
   - Datum vragen: 13-09-2016
   - Datum antwoorden: 17-10-2016
   - Gestelde vragen en antwoorden:

     Project Proposal:

     -3.2 De haalbaarheid is nog onvoldoende toegelicht en onderbouwd met referenties naar eigen werk.

     Antwoord: De haalbaarheid van het project is verder toegelicht in hoofdstuk 3.2 van het PP. De groep heeft verscheidene publicaties en veel ervaring met [redacted] *en* [redacted] *en het gebruik van* [redacted]*. Competentie voor het werken met* [redacted] *is*

vergaard aan de [redacted]. Kennis voor [redacted] in DM1 modellen wordt verzameld aan de [redacted]. Ervaring [redacted] injectie van [redacted] in [redacted] muizen zal worden opgedaan in het [redacted].

-3.4.1 De strategie voor het werk met [redacted] is duidelijk. De strategie-beschrijving van het werk met [redacted] is onvoldoende.

Antwoord: Wij zien inderdaad in dat de omschrijving van de humane experimenten nog niet helder was. De strategiebeschrijvingen van het werk met [redacted] zijn aangevuld in de hoofdstukken 3.1, 3.4.1 en 3.4.2 van het PP.

-3.4.2 Het muizenwerk vormt een afgerond geheel. Waarom is het nodig om ook het humane werk te doen? Zijn beide onderdelen van de projectaanvraag nodig voor het behalen van de doelstelling? Is er een volgorde in de experimenten, bijvoorbeeld eerst de rescue-experimenten in het DM1 muismodel alvorens de [redacted] in de [redacted] muizen worden onderzocht? De commissie mist een duidelijke beschrijving hiervan. Bovendien ontbreken de experimenten met [redacted] in de DAPs. Willen de onderzoekers overwegen dit project te beperken tot de experimenten met de DM1 muis?

Antwoord: Door zowel het muizen- als het humane werk te implementeren is het mogelijk de doelstelling van het project te behalen, namelijk het onderzoeken van de therapeutische waarde voor de patiënt. Verschillende vragen worden beantwoord door de twee uiteenlopende benaderingen:

1. *Ten eerste, door [redacted] uit [redacted] muis modellen, deze [redacted] en injecteren in [redacted] modellen is het mogelijk een functionele read-out van de behandeling toe te passen. Dit geeft een beeld van de effectiviteit van de behandeling.*
2. *Ten tweede, om te onderzoeken of deze aanpak therapeutische waarde heeft voor in de kliniek maken we gebruik van [redacted] en [redacted] deze, na [redacted], in [redacted] muizen. Zo kunnen we het gedrag van [redacted] in een [redacted] bestuderen.*

Het is op dit moment op basis van literatuurgegevens niet uit te sluiten dat [redacted] en [redacted] zich bij de [redacted] en [redacted]. Door gebruik te maken van beide benaderingen kunnen we in een zo kort mogelijke tijd efficiënt en met zo min mogelijk dieren een inschatting maken van de haalbaarheid van onze hypothese, om op termijn mogelijk iets voor patiënten te kunnen betekenen.

-3.4.3 Waarom zijn beide muismodellen noodzakelijk? Het [redacted] model is het enige model met een fenotype. Waarom is onderzoek in beide muismodellen nodig en kan niet worden volstaan met onderzoek in het [redacted] model?

Antwoord: Het includeren van beide muismodellen is van groot belang. Dit wordt in detail uitgelegd in hoofdstuk 3.4.3 van het PP.

[redacted] patiënten hebben een [redacted] in het [redacted]. Dit is een van de belangrijkste kenmerken van de aandoening. Hoe [redacted] de [redacted] is verschilt [redacted] van patiënt tot patiënt. Dit veroorzaakt verschil in ziektebeeld. [redacted] en [redacted] muizen hebben een [redacted] van [redacted]. [redacted] heeft een [redacted] en vertoont hierdoor ook een [redacted] fenotype. Het toepassen van de techniek op beide modellen geeft waardevolle informatie over de [redacted] Het laat zien of de [redacted], [redacted] en [redacted] mogelijk zijn in verschillende [redacted] modellen. Dit weerspiegelt de patiëntenpopulatie. Het zou fantastisch zijn als we met één techniek het hele patiëntenspectrum kunnen behandelen.

Description of Animal Procedures:

*DAP1

-A2: Het fenotype is niet goed uitgelegd voor beide modellen. Ook zijn de handelingen met de dieren niet adequaat beschreven.
Antwoord: De beschrijving van het fenotype en de handelingen met de [redacted] muizen zijn aangevuld. U kunt deze vinden in hoofdstuk 3.4.4.1 A.2.
-A3: De aantallen zijn niet onderbouwd (geldt ook voor andere DAPs). Waarom zijn er 200 [redacted] en 250 [redacted] dieren nodig? Optelling van de muizen opgevoerd in DAPs 2-4 leidt tot andere aantallen. Op p5 wordt gesteld dat 'Statistics, power calculations and pilot experiments are used to reduce the number of animals to an absolute minimal'. Het is de commissie volstrekt onduidelijk hoe deze intentie wordt waargemaakt.
Antwoord: Door de ervaring opgedaan aan de [redacted] zijn de aantallen zijn naar beneden bijgesteld. 150 [redacted] en 150 [redacted] muizen worden gefokt waarvan we er 100 includeren in [redacted] experimenten en 50 in [redacted] experimenten. In alle DAPs is de onderbouwing voor de aantallen uitgebreid.
-I: zacht voedsel werkt in zijn algemeenheid averechts bij [redacted].
Antwoord: [redacted] maar muizen die nog maar enkele dagen in het experiment blijven krijgen zacht voedsel (hoofdstukken 3.4.4.1 D.2 en 3.4.4.1 I.3). Op deze manier krijgen de dieren voldoende voedsel binnen maar hoeven ze niet de stressvolle procedure van [redacted] te ondergaan. Deze aanpak wordt in andere [redacted] fokken ook toegepast [redacted]).
-J: De CCD heeft geen toegang tot de genoemde intranetsite. Graag de te hanteren humane eindpunten beschrijven. (geldt ook voor DAP2)
Antwoord: Zoals omschreven in de eerste ingediende versie van DAP1 en DAP2 verwachten wij géén ongerief in de fok of weefsel isolatie waarbij het nodig is humane eindpunten te implementeren. Daarom wordt de algemene regelgeving aangehouden met humane eindpunten volgens 'OECD Guidance Document on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation' (OECD Guidance Document 19, 2000). Voor [redacted] kunt u de humane eindpunten die gehanteerd worden terug vinden in de hoofdstukken 3.4.4.3 J en 3.4.4.4 J.
-K: De percentages dieren die matig respectievelijk licht ongerief ondergaan ontbreken. Wat is het ongerief voor de dieren die binnen een maand na geboorte overlijden?
Antwoord: De percentages dieren die ongerief ondervinden in de [redacted] fok zijn terug te vinden in hoofdstuk 3.4.4.1 K. Het ongerief voor de [redacted] muizen die kort na geboorte overlijden is in de eerdere DEC geclassificeerd als mild (code 2), zo ook in deze aanvraag.
*DAP2
-B: Worden mannen en vrouwen in gelijke aantallen gebruikt? (geldt ook voor DAP3 en 4)?
Antwoord: De inclusie van mannen en vrouwen is 1:1 in DAP 2, 3 en 4
-B: Muizen ouder dan [redacted] worden gebruikt, maar hoe oud zijn de dieren maximaal bij gebruik voor een experiment? Het ongerief voor de [redacted] dieren is aanzienlijk.
Antwoord: Voor [redacted] experimenten is de optimale leeftijd [redacted] en de maximale leeftijd van de muizen [redacted] (hoofdstuk 3.4.4.2 B). Na [redacted] is de [redacted] van [redacted] moeilijk en inefficiënt. Omdat de dieren niet ouder worden dan [redacted] is er geen sprake van ongerief gerelateerd aan het fenotype. [redacted] en [redacted] van [redacted] beginnen pas vanaf [redacted] [redacted]).
-I en K: Het ongerief van het fenotype is niet meegenomen.
Antwoord: Er is geen sprake van het ongerief van het fenotype. Dit komt niet tot uiting voor [redacted]
*DAP3
-A1: tekst gaat over [redacted]). Gaat dit over [redacted] In het Project Proposal is geen sprake van muizen [redacted] Gaat het hier ineens over [redacted] die in [redacted] muizen dienen te worden [redacted] De laatste ontbreken volledig in de aanvraag.

Antwoord: Door [redacted] muizen [redacted] te injecteren in [redacted] muizen is het mogelijk een functionele read-out van de behandeling te onderzoeken. Dit geeft een idee over de effectiviteit van de behandeling. Door humane [redacted] te [redacted] in [redacted] muizen kunnen we in vivo het gedrag zien van de humane [redacted] en onderzoeken of de aanpak therapeutische waarde heeft voor de kliniek. Een aanvraag voor [redacted] van de [redacted] is ingediend bij de [redacted]. [redacted] van [redacted] cellen is mogelijk uit [redacted] materiaal van [redacted] maar nog nooit eerder geprobeerd bij [redacted] patiënten. Het is onduidelijk of uit een [redacted] voldoende [redacted] en [redacted] kunnen worden om de [redacted] therapie te testen. Met behulp van de [redacted]-techniek kunnen we oneindig veel humane [redacted] van lichaamscellen van [redacted] patiënten. We kunnen deze dan [redacted] muizen om het gedrag van de cellen in vivo te bestuderen (3.4.4.3 A1).
-A2: Hoe lang duurt het experiment maximaal? Worden de muizen meermaals functioneel getest? Worden ze direct na de functionele testen gedood?
Antwoord: Muizen worden getest vóór en na toediening van de [redacted] therapie. Functionele metingen worden gedaan met een maximum van 2 keer per week. De muizen worden gehouden tot een maximum leeftijd van 18 maanden. Na de laatste functionele test worden de muizen geofferd en wordt het weefsel geïsoleerd (hoofdstuk 3.4.4.3 A.2).
-B: Klopt het aantal dieren? In de figuur staat dat [redacted] muizen ook voor DAP4 worden gebruikt, maar deze komen in DAP4 niet meer voor. Zijn er dus 250 i.p.v. 500 dieren nodig? Deze dienen dan opgeteld te worden bij het totaal aantal benodigde dieren voor DAP3. Zie ook eerdere vraag over aantallen.
Antwoord: In DAP 3 en DAP 4 worden de [redacted] experimenten beschreven. Het aantal muizen dat nodig voor deze groep is eenmalig 50 [redacted] muizen en eenmalig 50 [redacted] muizen.
-I1 en K: niet alle oorzaken van ongerief zijn genoemd: experimentele handelingen, en fenotype ontbreken.
Antwoord: De informatie is aangevuld in hoofdstuk 3.4.4.3 I
-I3: maatregelen om ongerief van fenotype te minimaliseren ontbreken.
Antwoord: De informatie is aangevuld in hoofdstuk 3.4.4.3 I

- De antwoorden hebben geleid tot aanpassing van de aanvraag

- Datum vragen: 14-11-2016
- Datum antwoorden: 08-12-2016
- Gestelde vragen en antwoorden:

Description of Animal Procedures:
*DAP1
-K: het cumulatief ongerief voor de dieren is nog niet juist weergegeven. De onderzoekers worden verzocht hier het totale ongerief voor de dieren als gevolg van alle ongeriefsoorzaken (inclusief fenotype indien de dieren oud genoeg worden) samen te vermelden. Indien dit cumulatieve ongerief niet voor alle dieren hetzelfde is, dan dienen de percentages dieren vermeld te worden waarop dit ongerief betrekking heeft (samen 100%). (geldt ook voor de andere DAPs). Het ongerief dat wordt veroorzaakt door het doden zonder voorafgaande handelingen wordt in Nederland geclassificeerd als licht. De classificatie "terminaal" wordt alleen gebruikt wanneer eerst nog ingrepen onder anesthesie plaatsvinden, waarna het dier direct, onder dezelfde anesthesie, wordt gedood.
Antwoord: Per DAP is onderdeel K 'Classification of severity of procedure' aangepast. Wanneer het ongerief niet voor alle dieren hetzelfde is, wordt met percentages aangegeven op welke dieren het ongerief betrekking heeft.
*DAP2

-A: De onderzoekers zullen humane [redacted] in [redacted] muizen [redacted] Een beschrijving hiervan op p38/59 ontbreekt evenals een vermelding van de benodigde dieren onder B. Willen de onderzoekers inderdaad [redacted] muizen aanvragen?
Antwoord: Dat klopt. De [redacted] muizen die nodig zijn voor [redacted] van humane [redacted] worden gekocht (tabel DAP2 B blz. 15).
*DAP3
-B De aantallen dieren zijn nog niet navolgbaar voor de commissie. In de figuur bij onderdeel A wordt geen onderscheid gemaakt tussen dieren voor DAP2 en DAP3. De commissie verzoekt de onderzoekers in elke DAP duidelijk op te schrijven hoeveel dieren zij hiervoor aanvragen.
Antwoord: De afbeeldingen in DAP2 en 3 zijn verder uitgeschreven. 50 van de 50 [redacted] muizen, worden gebruikt voor [redacted] van [redacted] muis [redacted] zodat de [redacted] effecten kunnen worden gemeten. In 12 gekochte [redacted] muizen kan [redacted] het gedrag van herstelde humane [redacted] patiënten [redacted] worden getest. 36 van de 50 [redacted] dieren worden gebruikt voor [redacted]. De overige 14 [redacted] muizen ondergaan [redacted] toediening van de [redacted] therapie.

- De antwoorden hebben geleid tot aanpassing van de aanvraag.

10. Eventuele adviezen door experts (niet lid van de DEC):
    - Aard expertise
    - Deskundigheid expert
    - Datum verzoek
    - Strekking van het verzoek
    - Datum expert advies
    - Advies expert

B. Beoordeling (adviesvraag en behandeling)

1. Het project is vergunningplichtig (dierproeven in de zin der wet).
2. De aanvraag betreft een nieuwe aanvraag.
3. De DEC is competent om hierover te adviseren.
4. Er is geen betrokkenheid van DEC-leden bij deze projectaanvraag, waardoor onafhankelijkheid en onpartijdigheid zijn gewaarborgd.

C. Beoordeling (inhoud)

1. Deze aanvraag heeft een concrete doelstelling en kan getypeerd worden als een project. De opzet komt het best overeen met voorbeeld 4B uit de handreiking 'Invulling definitie project' van de CCD. Het is helder welke handelingen individuele dieren zullen ondergaan. Hierdoor is ook duidelijk welk ongerief individuele dieren zullen ondergaan. De aanvrager heeft, zowel binnen de doelstellingen en bijlagen dierproeven, als tussen de doelstellingen, beschreven op basis van welke criteria zij zal besluiten het project wel of niet te continueren. De DEC is er daardoor van overtuigd dat de aanvrager gedurende het project op zorgvuldige wijze besluiten zal nemen over de voortgang van het project en dat er niet onnodig dieren gebruikt zullen worden.

2. Voor zover de DEC weet is er geen "tegenstrijdige" wetgeving die het uitvoeren van de proef in de weg zou kunnen staan.

3. De in de aanvraag aangekruiste doelcategorie is in overeenstemming met de hoofddoelstelling.

Belangen en waarden

4. Het directe doel van het project is te onderzoeken of [redacted] of [redacted] gebruikt kunnen worden als [redacted] voor [redacted] in muismodellen voor deze aandoening. Het uiteindelijke doel is de ontwikkeling van een therapie die de [redacted] van [redacted] bij DM1 patiënten tegengaat door het toedienen van [redacted] of [redacted]. Behandeling met [redacted] wordt al uitgetest bij andere spierziekten [redacted]). De techniek waarmee het [redacted] kan worden hersteld ([redacted])wordt in vele labs met succes gebruikt. De DEC is daarom van mening dat er binnen dit project een directe relatie is tussen het korte termijn doel en het uiteindelijke doel. De DEC is bovendien van mening dat het directe doel gerechtvaardigd is binnen de context van het onderzoeksveld.

5. De belangrijkste belanghebbenden in deze projectaanvraag zijn de proefdieren, de onderzoekers en de doelgroep/patiënten.
   Voor de proefdieren geldt dat hun welzijn en integriteit worden aangetast. De dieren zullen beperkt worden in hun natuurlijke gedrag en gedurende de proeven zullen de dieren stress ondervinden en pijn ondergaan. Uiteindelijk zullen ze in het kader van het onderzoek gedood worden. De dieren hebben er belang bij hiervan gevrijwaard te blijven.
   Voor de onderzoekers geldt dat het publiceren van belangrijke nieuwe wetenschappelijke inzichten resulteert in een goede wetenschappelijke reputatie, hetgeen vaak de sleutel is voor het verkrijgen van nieuwe onderzoeksmogelijkheden. Carrièremogelijkheden en welstand kunnen door de onderzoeker zelf van belang geacht worden, maar dienen naar de mening van de DEC geen rol te spelen in de ethische afweging over de toelaatbaarheid van het gebruik van proefdieren. Het gaat uiteindelijke om de vraag of dit onderzoek belangrijke maatschappelijke en wetenschappelijke doelen dient (gezondheid, kennis). Er dient tenminste (ook) sprake te zijn van een algemeen of publiek belang, wil een dierproef gerechtvaardigd zijn.
   Voor patiënten is dit onderzoek van belang, omdat het kan bijdragen aan een verbetering van hun gezondheid en kwaliteit van leven. Gerichte behandeling op basis van mechanistisch inzicht kan bijdragen aan een betere behandeling met minder bijwerkingen. Dit kan er toe leiden dat de patiënt een betere kwaliteit van leven heeft. Kunnen beschikken over adequate behandelingen voor ernstige ziekten, zoals DM1, is van groot belang voor de samenleving.

6. De onderzoekers maken gebruik van transgene dieren waarbij zij de nationale GGO-regels in acht nemen. Hierdoor is er geen sprake van belangwekkende milieueffecten.

Proefopzet en haalbaarheid

7. De kennis en kunde van de onderzoeksgroep en andere betrokkenen bij de dierproeven zijn voldoende gewaarborgd. De commissie is overtuigd van de kwaliteit van het werk van de aanvrager, zoals blijkt uit de in de aanvraag vermelde publicaties van deze onderzoeksgroep. De aanvragers beschikken over voldoende kennis en kunde om te kunnen voldoen aan alle zorgvuldigheidseisen omtrent het verrichten van dierproeven.

8. De doelstellingen van het project zijn realistisch en de voorgestelde experimentele opzet en uitkomstparameters sluiten hier logisch bij aan. Bovendien heeft deze groep veel ervaring in dit onderzoeksveld en met de voorgestelde dierproeven. De DEC is dan ook van mening dat het project goed is opgezet, en dat deze strategie en experimentele aanpak kunnen leiden tot het behalen van de doelstelling binnen het kader van het project.

Welzijn dieren

9. Er is geen sprake van de volgende bijzonderheden op het gebied van categorieën van dieren, omstandigheden of behandeling van de dieren:
   ☐ Bedreigde diersoort(en) (10e lid 4)
   ☐ Niet-menselijke primaten (10e)
   ☐ Dieren in/uit het wild (10f)
   ☐ Niet gefokt voor dierproeven (11, bijlage I richtlijn)
   ☐ Zwerfdieren (10h)
   ☐ Hergebruik (1e lid 2)
   ☐ Locatie: buiten instelling vergunninghouder (10g)
   ☐ Geen toepassing verdoving/pijnbestrijding (13)
   ☐ Dodingsmethode niet volgens bijlage IV richtlijn (13c lid 3)

10. De huisvesting en verzorging van de dieren zijn conform de eisen in bijlage III van richtlijn 2010/63/EU.

11. Het cumulatieve ongerief als gevolg van de dierproeven is realistisch ingeschat en geclassificeerd. Het ongerief wordt hoofdzakelijk veroorzaakt door de perinatale sterfte, [redacted] bij sommige dieren, en het [redacted] door dieren die [redacted] hebben. Het cumulatief ongerief is juist ingeschat als licht voor 30% van de dieren en matig voor 70 % van de dieren.

12. De integriteit van dieren is aangetast doordat zij [redacted] zijn. Deze [redacted] leidt tot [redacted] en op latere leeftijd tot [redacted] Op zichzelf kan het “inbouwen” van een dergelijk [redacted] als een vrij ernstige aantasting van de integriteit worden beschouwd. Deze [redacted] heeft echter geen gevolgen voor de zelfredzaamheid van de dieren tijdens de duur van het experiment. Daar niet de intentie bestaat de dieren in leven te laten tot het [redacted] tot expressie komt, kiest de commissie er voor dit mee te wegen op dezelfde wijze als andere [redacted] die niet tot een afwijkend fenotype leiden.

13. De criteria voor humane eindpunten zijn voldoende specifiek gedefinieerd en toegesneden op het experiment. Het percentage dieren dat naar verwachting een humaan eindpunt zal bereiken is op basis van eerdere ervaringen met de diermodellen ingeschat. De commissie is het dan ook eens met deze inschatting en de gehanteerde humane eindpunten.

3V’s

14. De aanvrager heeft voldoende aannemelijk gemaakt dat er geen geschikte vervangingsalternatieven zijn. Het effect van [redacted] bij DM1 kan alleen goed in een proefdiermodel met dezelfde [redacted] die DM1 veroorzaakt onderzocht worden. De onderdelen van het project die in vitro bestudeerd kunnen worden zijn al uitgevoerd. Voor het beantwoorden van de resterende onderzoeksvragen zijn dierproeven noodzakelijk.

15. Het maximale aantal te gebruiken dieren is realistisch ingeschat en is proportioneel ten opzichte van de gekozen onderzoeksopzet en de looptijd. De onderzoekers hanteren een goede strategie om ervoor te zorgen dat er met zo min mogelijk dieren wordt gewerkt waarmee een

wetenschappelijk betrouwbaar resultaat kan worden verkregen. Door de stapsgewijze aanpak waarin de resultaten uit eerdere proeven worden gebruikt voor het design van vervolgexperimenten wordt onnodig gebruik van proefdieren voorkomen. De commissie heeft met de aanvragers gediscussieerd over de noodzaak van het gebruik van meer dan één diermodel. De beide [redacted] diermodellen vormen samen een afspiegeling van de patiëntenpopulatie, waardoor de DEC overtuigd is van het belang van het gebruik van beide modellen. De experimenten met de [redacted] dieren zullen duidelijk maken of de beoogde [redacted] bij patiënten succesvol kan zijn.

16. Het project is in overeenstemming met de vereiste van de verfijning van dierproeven. De muis is de minst complexe diersoort met skeletspieren die voldoende overeenkomen met de humane situatie. Bovendien is er een muismodel beschikbaar voor deze ziekte. De onderzoekers beogen een optimale behandeling met zo min mogelijk [redacted] De DEC is ervan overtuigd dat de beschreven proefopzet de meest verfijnde is en dat de dierproeven zo humaan mogelijk worden uitgevoerd.

17. Het betreft geen wettelijk vereist onderzoek.

    Dieren in voorraad gedood en bestemming dieren na afloop proef

18. Dieren van beide geslachten zullen in gelijke mate ingezet worden.

19. De dieren zullen in het kader van het project gedood worden. Dit is noodzakelijk om spierweefsels te kunnen onderzoeken voor het beantwoorden van bepaalde onderzoeksvragen. De gebruikte dodingsmethode staat vermeld in bijlage IV van richtlijn 2010/63/EU.

20. Er worden in deze projectaanvraag geen landbouwhuisdieren, honden, katten of niet-humane primaten gedood om niet-wetenschappelijke redenen.

    NTS

21. De niet-technische samenvatting is een evenwichtige weergave van het project en begrijpelijk geformuleerd.

D. Ethische afweging

1. Rechtvaardigt het belang van de doelstelling van het project het ongerief dat de dieren wordt aangedaan, en is aan alle zorgvuldigheidseisen (3V's) voldaan?

2. Er vindt een lichte of matige aantasting van welzijn en integriteit van de proefdieren plaats (beschreven in C9 tot C20). De doelstellingen kunnen niet zonder dieren behaald worden. De onderzoekers doen al het mogelijke om het lijden van de dieren en het aantal dieren te beperken.
   Voor patiënten is dit onderzoek van belang, omdat het op termijn kan bijdragen aan een verbetering van hun gezondheid en kwaliteit van leven. De DEC kent daar veel gewicht aan toe. DM1 is een ernstige progressieve erfelijke ziekte die ondermeer leidt tot voortdurende afname van [redacted] waardoor de patiënt steeds verder beperkt wordt in zijn [redacted] en een beperkte levensverwachting heeft. Het is aannemelijk dat de doelstellingen op termijn behaald zullen worden. De commissie acht het ontwikkelen van een [redacted] waarmee de [redacted].

3. De DEC is overtuigd van het belang van de doelstellingen: onderzoeken of [redacted] gebruikt kunnen worden als [redacted]) in muismodellen voor deze aandoening. Het uiteindelijke doel daarvan is de ontwikkeling van een therapie die de [redacted]l bij DM1 patiënten tegengaat. De DEC is van mening dat de belangen van de patiënten voldoende zwaar wegen om het schaden van de belangen van de proefdieren (om gevrijwaard te blijven van een aantasting van hun welzijn en integriteit) te rechtvaardigen. De commissie is overtuigd van de kwaliteit van het werk van de aanvrager. De DEC is van mening dat het project goed is opgezet, en dat de gekozen strategie en experimentele aanpak kunnen leiden tot het behalen van de doelstelling binnen het kader van het project. De aanvrager heeft voldoende aannemelijk gemaakt dat er geen geschikte vervangingsalternatieven zijn, dat het doel niet met minder dieren behaald kan worden, dat de gebruikte aanpak de meest verfijnde is en dat zij zal kunnen voorkomen dat mens, dier en het milieu onbedoelde negatieve effecten ondervinden als gevolg van de dierproeven.
   De DEC is van oordeel dat het hier boven geschetste belang de onvermijdelijke nadelige gevolgen van dit onderzoek voor de dieren, in de vorm van angst, pijn of stress, rechtvaardigt. Aan de eis dat het belang van de experimenten op dient te wegen tegen het ongerief dat de dieren wordt berokkend, is voldaan.

E. Advies

1. Advies aan de CCD
   ☒ De DEC adviseert de vergunning te verlenen

   ☐ De DEC adviseert de vergunning te verlenen onder de volgende voorwaarden
      - Op grond van het wettelijk vereiste dient de projectleider bij beëindiging van het project een beoordeling achteraf aan te leveren die is afgestemd met de IvD.
      - Voor de uitvoering van dit project is tevens ministeriële ontheffing vereist
      - Overige door de DEC aan de uitvoering verbonden voorwaarden, te weten...

   ☐ De DEC adviseert de vergunning niet te verlenen vanwege:
      - De vaststelling dat het project niet vergunningplichtig is om de volgende redenen:…
      - De volgende doorslaggevende ethische bezwaren:…
      - De volgende tekortkomingen in de aanvraag:…

2. Het uitgebrachte advies is gebaseerd op consensus.

3. Er zijn geen knelpunten of dilemma's geconstateerd – zowel binnen als buiten de context van het project - die de verantwoordelijkheid en competentie van de DEC overstijgen.

Centrale Commissie Dierproeven

11

> Retouradres Postbus 20401 2500 EK Den Haag

Stichting Katholieke Universiteit Nijmegen
Instantie voor dierenwelzijn
Postbus 9101, t.a.v. [redacted]
6500 HB NIJMEGEN
[redacted]

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD103002016801

Bijlagen
2

Datum 22 december 2016
Betreft Ontvangstbevestiging aanvraag projectvergunning Dierproeven

Geachte [redacted]

Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 22 december 2016. Het gaat om uw project "Development of a cell therapy against the muscular phenotype of Myotonic Dystrophy ". Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD103002016801. Gebruik dit nummer wanneer u contact met de CCD opneemt.

Wacht met de uitvoering van uw project
Als wij nog informatie van u nodig hebben dan ontvangt u daarover bericht. Uw aanvraag is in ieder geval niet compleet als de leges niet zijn bijgeschreven op de rekening van de CCD. U ontvangt binnen veertig werkdagen een beslissing op uw aanvraag. Als wij nog informatie van u nodig hebben, wordt deze termijn opgeschort. In geval van een complexe aanvraag kan deze termijn met maximaal vijftien werkdagen verlengd worden. U krijgt bericht als de beslisperiode van uw aanvraag vanwege complexiteit wordt verlengd. Als u goedkeuring krijgt op uw aanvraag, kunt u daarna beginnen met het project.

Factuur
Bijgaand treft u de factuur aan voor de betaling van de leges. Wij verzoeken u de leges zo spoedig mogelijk te voldoen, zodat we uw aanvraag in behandeling kunnen nemen. Is uw betaling niet binnen dertig dagen ontvangen, dan kan uw aanvraag buiten behandeling worden gesteld. Dit betekent dat uw aanvraag niet beoordeeld wordt en u uw project niet mag starten.

Meer informatie
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Datum:
22 december 2016
Aanvraagnummer:
AVD103002016801

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlagen:
- Gegevens aanvraagformulier
- Factuur

Datum:
22 december 2016
Aanvraagnummer:
AVD103002016801

Gegevens aanvrager

Uw gegevens	
Deelnemersnummer NVWA:	10300
Naam instelling of organisatie:	Stichting Katholieke Universiteit Nijmegen
KvK-nummer:	41055629
Postbus:	9101, t.a.v. [redacted]
Postcode en plaats:	6500 HB NIJMEGEN
IBAN:	NL90ABNA0231209983
Tenaamstelling van het rekeningnummer:	UMC St Radboud

Gegevens verantwoordelijke onderzoeker	
Naam:	[redacted]
Functie:	[redacted]
Afdeling:	[redacted]
Telefoonnummer:	[redacted]
E-mailadres:	[redacted]

Datum:
22 december 2016
Aanvraagnummer:
AVD103002016801

Gegevens plaatsvervangende verantwoordelijke onderzoeker

Naam:	[redacted]
Functie:	[redacted]
Afdeling:	[redacted]
Telefoonnummer:	[redacted]
E-mailadres:	[redacted]

Gegevens verantwoordelijke uitvoering proces

Functie:	Instantievoor Dierenwelzijn
Telefoonnummer:	[redacted]
E-mailadres:	[redacted]

Gegevens gemachtigde

Naam:	[redacted]
Postcode en plaats:	6500 HB NIJMEGEN
Wilt u een nieuwe machtiging afgeven?	Nee
Wat mag de gemachtigde doen?	[ ] Een projectvergunning aanvragen [ ] Een wijziging op een verleende projectvergunning aanvragen [ ] Een melding doorgeven op een verleende projectvergunning [ ] Een bezwaarschrift indienen en daarover communiceren met de Centrale Commissie Dierproeven en alle andere handelingen verrichten die nodig zijn voor een goede afwikkeling van het bezwaarschrift [x] Alle bovenstaande opties

Over uw aanvraag

Wat voor aanvraag doet u?	[x] Nieuwe aanvraag [ ] Wijziging op een (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn [ ] Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn

Datum:
22 december 2016
Aanvraagnummer:
AVD103002016801

Over uw project

Geplande startdatum:	22 januari 2017
Geplande einddatum:	22 januari 2022
Titel project:	Development of a cell therapy against the muscular phenotype of Myotonic Dystrophy
Titel niet-technische samenvatting:	Ontwikkeling van een celtherapie voor Myotone Dystrofie type 1
Naam DEC:	RU DEC
Postadres DEC:	Postbus 9101, 6500 HB Nijmegen [redacted]
E-mailadres DEC:	[redacted]

Betaalgegevens

De leges bedragen:	€ 1.441,-
De leges voldoet u:	na ontvangst van de factuur

Checklist bijlagen

Verplichte bijlagen:	[x] Projectvoorstel [x] Beschrijving Dierproeven [x] Niet-technische samenvatting
Overige bijlagen:	[x] Melding Machtiging [x] DEC-advies

Ondertekening

Naam:	[redacted]
Functie:	Instantie voor dierenwelzijn
Plaats:	Nijmegen
Datum:	22 december 2016

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Instantie voor dierenwelzijn
Postbus 9101, t.a.v. [redacted]
6500 HB NIJMEGEN
[redacted]

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD103002016801
Bijlagen
2

Datum 22 december 2016
Betreft Factuur aanvraag projectvergunning Dierproeven

Factuur
Factuurdatum: 22 december 2016
Vervaldatum: 21 januari 2017
Factuurnummer: 16700801
Ordernummer: 040823-461220/2016-0049/ [redacted]

Omschrijving	**Bedrag**
Betaling leges projectvergunning dierproeven Betreft aanvraag AVD103002016801	€ 1.441,00

Wij verzoeken u het totaalbedrag vóór de gestelde vervaldatum over te maken op rekening NL29INGB 070.500.1512 onder vermelding van het factuurnummer en aanvraagnummer, ten name van Centrale Commissie Dierproeven, Postbus 93144, 2509 AC te 's Gravenhage.

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Stichting Katholieke Universiteit Nijmegen
Instantie voor Dierenwelzijn
t.a.v. [redacted],
Postbus 9101, [redacted]
6500 HB (628) NIJMEGEN
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Centrale Commissie Dierproeven

Postbus 20401
2500 EK Den Haag
www.centrale commissiedierproeven.nl

T 0900-28 000 28 (10 ct /min)

info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD103002016801

Uw referentie

Bijlagen
1

Datum 6 januari 2017
Betreft Aanvulling Aanvraag projectvergunning dierproeven

Geachte [redacted],

Op 22 december 2016 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Development of a cell therapy against the muscular phenotype of Myotonic Dystrophy" met aanvraagnummer AVD103002016801. In uw aanvraag zitten voor ons nog enkele onduidelijkheden. In deze brief leest u wat wij nog nodig hebben en wanneer u een beslissing kunt verwachten.

Welke informatie nog nodig
Wij hebben de volgende informatie van u nodig om uw aanvraag verder te kunnen beoordelen:

1) In uw aanvraag geeft u aan muizen te willen gebruiken. De CCD hecht er aan dat het aantal dieren in voorraad gedood terug te dringen. Kunt u toelichten of het dieren van beide geslachten worden gebruiken en als dit niet mogelijk is kunt u dan onderbouwen waarom het belangrijk is dieren van 1 geslacht te gebruiken?

2) In bijlage dierproeven 3.4.4.1 meldt u dat 'Optimization of the procedure is established with [redacted] and wt littermates born from [redacted] breeding.' Onder punt 'B. The animals' geeft u alleen het aantal [redacted] muizen aan. Het is voor ons niet duidelijk hoe veel dieren voor de optimalisatie van de procedures worden gebruikt en waarom deze dieren niet meegerekend zijn in het aantal benodigde dieren. Zou u dit willen toelichten en indien van toepassing graag in uw aanvraag aanpassen?

3) In de bijlage dierproeven 3.4.4.3 beschrijft u ook pilotexperimenten te willen uitvoeren met n=3 dieren. Het is voor ons niet duidelijk of deze dieren meegerekend zijn in het aantal vermelde [redacted] dieren (35 [redacted] muizen). Zou u dit willen toelichten en indien van toepassing graag het aantal dieren in uw aanvraag aanpassen?

4) In het projectvoorstel beschrijft u [redacted] applicatie van [redacted] te willen uitvoeren. Begrijpen we goed dat dit experiment onder de bijlage dierproeven 3.4.4.3 valt?
In de figuur op pagina 12 van het projectvoorstel geeft u aan dat '[redacted]' met de [redacted] ook in [redacted] muizen worden gedaan.
Indien dit experiment onder bijlage 3.4.4.3 valt is voor ons niet duidelijk hoe veel [redacted] muizen hiervoor worden ingezet. In deze bijlage wordt niet naar de [redacted] muizen verwezen. Kunt u dit uitleggen en indien nodig de aanvraag aanpassen?

Opsturen binnen veertien dagen
Stuur de ontbrekende informatie binnen veertien dagen na de datum van deze brief op. U kunt dit aanleveren via NetFTP. Gebruik hierbij het formulier dat u bij deze brief krijgt indien u uw antwoord per post verstuurt.

Wanneer een beslissing
De behandeling van uw aanvraag wordt opgeschort tot het moment dat wij de aanvullende informatie hebben ontvangen. Uw aanvraag is in ieder geval niet compleet als de leges niet zijn ontvangen. Als u goedkeuring krijgt op uw aanvraag, kunt u daarna beginnen met het project.

Meer informatie
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlage:
- formulier Melding Bijlagen via de post

Centrale Commissie Dierproeven

Melding

Bijlagen via de post

- U wilt één of meerdere bijlagen naar ons versturen? Voeg *altijd* deze Melding Bijlagen toe. Wij weten dan welke documenten van u zijn en hoeveel documenten u opstuurt.
- Meer informatie vindt u op www.zbo-ccd.nl
- Of bel met ons: 0900 28 000 28 (10 ct/min).

1 Uw gegevens

1.1 Vul de gegevens in.

Naam aanvrager			
Postcode		Huisnummer	

1.2 Bij welke aanvraag hoort de bijlage?
Het aanvraagnummer staat in de brief of de ontvangstbevestiging.

Aanvraagnummer	

2 Bijlagen

2.1 Welke bijlagen stuurt u mee?
Vul de naam of omschrijving van de bijlage in.

☐	
☐	
☐	

3 Ondertekening

3.1 Onderteken het formulier en stuur het met alle bijlagen op naar:

Centrale Commissie
Dierproeven
Postbus 20401
2500 EK Den Haag

Naam	
Datum	- - 20
Handtekening	

[redacted])

Van: Info-zbo
Verzonden: dinsdag 10 januari 2017 16:54
Aan: [redacted]
CC: [redacted]
Onderwerp: Extra aanvullende informatie aanvraag AVD103002016801

Geachte [redacted]

In aanvulling op de eerder gevraagde aanvullende informatie willen we u graag vragen om de tekst onder kopje 'D. Replacement, reduction, refinement' in alle bijlages dierproeven verder uit te werken. De beschrijving van de toepassing van de methoden voor vervanging, vermindering en verfijning is summier. Vooral de methoden voor vervanging ontbreken in uw aanvraag.

Om uw aanvraag in de eerstkomende CCD vergadering te kunnen bespreken ontvangen we graag uw antwoord uiterlijk maandag, 16 januari 2017.

De behandeling van uw aanvraag wordt opgeschort tot het moment dat wij de aanvullende informatie hebben ontvangen.

Met vriendelijke groet,

[redacted]
Uitvoeringsexpert

Centrale Commissie Dierproeven **www.centralecommissiedierproeven.nl**
Postbus 20401 | 2500 EK | Den Haag
T: 0900 2800028
E: info@zbo-ccd.nl

Van: Info-zbo
Verzonden: vrijdag 6 januari 2017 12:22
Aan: [redacted]
CC: [redacted]
Onderwerp: aanvullende informatie aanvraag AVD103002016801

Geachte heer Sneepers,

Op 22 december 2016 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Development of a cell therapy against the muscular phenotype of Myotonic Dystrophy" met aanvraagnummer AVD103002016801. In uw aanvraag zitten voor ons nog enkele onduidelijkheden. In de bijgaande brief leest u wat wij nog nodig hebben en wanneer u een beslissing kunt verwachten.

Stuur de ontbrekende informatie binnen veertien dagen na de datum van deze brief op. Om uw aanvraag in de eerstkomende CCD vergadering te kunnen bespreken ontvangen we graag uw antwoord uiterlijk **maandag, 16 januari 2017**.

De behandeling van uw aanvraag wordt opgeschort tot het moment dat wij de aanvullende informatie hebben ontvangen.

Met vriendelijke groet,

[redacted]
Uitvoeringsexpert

Centrale Commissie Dierproeven **www.centralecommissiedierproeven.nl**
Postbus 20401 | 2500 EK | Den Haag

T: 0900 2800028
E: info@zbo-ccd.nl

[redacted])

Van: [redacted]
Verzonden: maandag 9 januari 2017 12:33
Aan: info@zbo-ccd.nl
CC: dierexperimentencommissie@radboudumc.nl
Onderwerp: RE: aanvullende informatie aanvraag AVD103002016801

Categorieën: Dossier: [redacted]

Geachte [redacted],

Allereerst ook voor u en de andere medewerkers van het bureau van de CCD de beste wensen voor 2017!

Excuses voor de onduidelijkheden in het DEC-advies wat betreft het aantal bijlagen en het gebruik van mannelijke en vrouwelijke dieren.

1. De aanvrager heeft tussentijds besloten (na overleg met de IvD) om de eerste bijlage (DAP1)weg te laten. Hierin werd alleen de fok voor de benodigde dieren beschreven. Deze fok is nu toegevoegd aan de drie bijlages waarin de experimenten met de dieren worden beschreven, overeenkomstig de richtlijnen van de CCD. De nummering van de bijlages is aangepast (wat eerst DAP4 was is nu DAP3 etc.).
2. De aanvrager heeft in eerste instantie het geslacht van de dieren niet vermeld. Volgens het format DEC-advies dient de DEC advies te geven over het gebruik van mannelijke en/of vrouwelijke dieren. Na de vraag van de DEC hierover heeft de onderzoeker geantwoord dat dieren van beide geslachten in gelijke aantallen gebruikt zullen worden in de experimenten. Deze informatie is opgenomen in het DEC-advies, maar kennelijk niet verwerkt in de projectaanvraag.

Ik hoop uw vragen aan de DEC hiermee voldoende te hebben beantwoord. Indien u meer informatie nodig heeft of een nieuw dec-advies waarin deze antwoorden zijn verwerkt, dan hoor ik het graag.

Met vriendelijke groet,
[redacted]
[redacted]

Van: Info-zbo [mailto:info@zbo-ccd.nl]
Verzonden: vrijdag 6 januari 2017 12:43
Aan: Postbus DierExperimenten Commissie
Onderwerp: aanvullende informatie aanvraag AVD103002016801

Geachte RUDEC,
Beste wensen voor 2017!
Op 21 december 2016 heeft u advies uitgebracht op een projectaanvraag met titel 'Development of a cell therapy against the muscular phenotype of Myotonic Dystrophy type 1'en aanvraagnummer AVD103002016801, uw kenmerk 2016-0049.
In uw advies zitten voor ons nog enkele onduidelijkheden.
1) In de beschrijving van uw correspondentie met de aanvrager werd enkele keren naar DAP 4 gerefereerd: *'Optelling van de muizen opgevoerd in DAPs 2-4 leidt tot andere aantallen.'; '-B: Worden mannen en vrouwen in gelijke aantallen gebruikt? (geldt ook voor DAP3 en 4)? Antwoord: De inclusie van mannen en vrouwen is 1:1 in DAP 2, 3 en 4.'; '-B: Klopt het aantal dieren? In de figuur staat dat [redacted] muizen ook voor DAP4 worden gebruikt, maar deze komen in DAP4 niet meer voor. Zijn er dus 250 i.p.v. 500 dieren nodig? Deze dienen dan opgeteld te worden bij het totaal aantal benodigde dieren voor DAP3. Zie ook eerdere vraag over aantallen. Antwoord: In DAP 3 en DAP 4 worden de [redacted] experimenten beschreven. Het aantal muizen dat nodig voor deze groep is eenmalig 50 [redacted] muizen en eenmalig 50 [redacted] muizen.'*
In de aanvraag die wij hebben ontvangen zijn er maar 3 bijlages dierproeven benoemd en beschreven. Het is voor ons niet duidelijk over welke 4 bijlages dierproeven wordt in uw advies besproken. Zou u dit punt kunnen verhelderen?

2) In de aanvraag wordt het geslacht van de muizen niet benoemd. Daarom is ook de volgende correspondentie tussen u en de aanvrager voor ons een beetje verwarrend: '-B: Worden mannen en vrouwen in gelijke aantallen gebruikt? (geldt ook voor DAP3 en 4)? Antwoord: De inclusie van mannen en vrouwen is 1:1 in DAP 2, 3 en 4.' Heeft u meer informatie hierover, of hoort deze correspondentie per ongeluk bij een andere aanvraag/advies? Zou u hierop uiterlijk maandag, 16 januari 2017, willen reageren?
De volgende vragen zijn aan de aanvrager voorgelegd:
1) In uw aanvraag geeft u aan muizen te willen gebruiken. De CCD hecht er aan dat het aantal dieren in voorraad gedood terug te dringen. Kunt u toelichten of het dieren van beide geslachten worden gebruiken en als dit niet mogelijk is kunt u dan onderbouwen waarom het belangrijk is dieren van 1 geslacht te gebruiken?
2) In bijlage dierproeven 3.4.4.1 meldt u dat 'Optimization of the procedure is established with [redacted] and wt littermates born from [redacted] breeding.' Onder punt 'B. The animals' geeft u alleen het aantal [redacted] muizen aan. Het is voor ons niet duidelijk hoe veel dieren voor de optimalisatie van de procedures worden gebruikt en waarom deze dieren niet meegerekend zijn in het aantal benodigde dieren. Zou u dit willen toelichten en indien van toepassing graag in uw aanvraag aanpassen?
3) In de bijlage dierproeven 3.4.4.3 beschrijft u ook pilotexperimenten te willen uitvoeren met n=3 dieren. Het is voor ons niet duidelijk of deze dieren meegerekend zijn in het aantal vermelde [redacted] dieren (35 [redacted] muizen). Zou u dit willen toelichten en indien van toepassing graag het aantal dieren in uw aanvraag aanpassen?
4) In het projectvoorstel beschrijft u [redacted]) applicatie van [redacted] te willen uitvoeren. Begrijpen we goed dat dit experiment onder de bijlage dierproeven 3.4.4.3 valt?
In de figuur op pagina 12 van het projectvoorstel geeft u aan dat [redacted]' met de [redacted] [redacted] ook in [redacted] muizen worden gedaan.
Indien dit experiment onder bijlage 3.4.4.3 valt is voor ons niet duidelijk hoe veel naakte muizen hiervoor worden ingezet. In deze bijlage wordt niet naar de [redacted] muizen verwezen. Kunt u dit uitleggen en indien nodig de aanvraag aanpassen?
Indien u ook hierop wil reageren, horen we het graag.
Alvast hartelijk dank.
Met vriendelijke groet,

[redacted]
Uitvoeringsexpert
Centrale Commissie Dierproeven **www.centralecommissiedierproeven.nl**
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Centrale Commissie Dierproeven
Postbus 20401
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13 januari 2016

Kenmerk: **aanvraagnummer AVD103002016801**

Betreft: Aanvulling aanvraag projectvergunning dierproeven

Geachte leden van de Centrale Commissie Dierproeven,

Hiermee verzoeken wij de leden van de Centrale Commissie Dierproeven om een beoordeling van het onderzoek getiteld '*Development of a cell therapy against the muscular phenotype of Myotonic Dystrophy type 1*', geregistreerd onder nummer AVD103002016801.

Onderstaand vindt u de reactie op uw vragen en opmerkingen. De nieuwe rapporten van de herziene projectbeschrijving staan in iVentionLES. Wij hopen u hiermee voldoende geïnformeerd te hebben.

Met vriendelijke groet,

[redacted]
Radboudumc Nijmegen
Department of Human Genetics
Geert Grooteplein-[redacted]
6525 GA Nijmegen
The Netherlands
[redacted]

[redacted]
Radboudumc Nijmegen
Department of [redacted]
Geert Grooteplein [redacted]
6525 GA Nijmegen
The Netherlands
[redacted]

De CCD hecht er aan dat het aantal dieren in voorraad gedood terug te dringen. Kunt u toelichten of het dieren van beide geslachten worden gebruiken en als dit niet mogelijk is kunt u dan onderbouwen waarom het belangrijk is dieren van 1 geslacht te gebruiken?

In onze aanvraag worden dieren van beide geslachten gebruikt.

In bijlage dierproeven 3.4.4.1 meldt u dat 'Optimization of the procedure is established with [redacted] and [redacted] littermates born from [redacted] breeding.' Onder punt 'B. The animals' geeft u alleen het aantal [redacted] muizen aan. Het is voor ons niet duidelijk hoe veel dieren voor de optimalisatie van de procedures worden gebruikt en waarom deze dieren niet meegerekend zijn in het aantal benodigde dieren. Zou u dit willen toelichten en indien van toepassing graag in uw aanvraag aanpassen?

Onze excuses voor deze omissie. Het aantal dieren dat gebruikt wordt voor de optimalisatie is in de nieuwe versie van de aanvraag weergegeven in figuur 1 onder punt 3.4.4.1 B 'The animals'. Het gaat om 10% van de wt en [redacted] dieren. Deze surplus dieren zijn 'overtallig' van de [redacted] fok en zouden anders ook geëuthaniseerd worden. Het aantal is hoger voor de [redacted] dieren aangezien onze focus voornamelijk ligt op het [redacted] muismodel maar ook validatie en optimalisatie voor de [redacted] muizen is nodig omdat het verschil [redacted] achtergrond een effect kan hebben op cel isolatie.

In de bijlage dierproeven 3.4.4.3 beschrijft u ook pilot-experimenten te willen uitvoeren met n=3 dieren. Het is voor ons niet duidelijk of deze dieren meegerekend zijn in het aantal vermelde [redacted] dieren (35 [redacted] muizen). Zou u dit willen toelichten en indien van toepassing graag het aantal dieren in uw aanvraag aanpassen?

De dieren geïncludeerd in het pilot experimenten waren nog niet meegerekend in het totaal aantal [redacted] dieren. Dit is nu aangepast in 3.4.4.3. B 'The animals'.

In het projectvoorstel beschrijft u [redacted] applicatie van [redacted] te willen uitvoeren. Begrijpen we goed dat dit experiment onder de bijlage dierproeven 3.4.4.3 valt?

Ja, de [redacted] toediening valt onder bijlage 3.4.4.3. '[redacted] application of [redacted]

In de figuur op pagina 12 van het projectvoorstel geeft u aan dat [redacted] met de geïsoleerde cellen ook in [redacted] muizen worden gedaan. Indien dit experiment onder bijlage 3.4.4.3 valt is voor ons niet duidelijk hoe veel [redacted] muizen hiervoor worden ingezet. In deze bijlage wordt niet naar de [redacted] muizen verwezen. Kunt u dit uitleggen en indien nodig de aanvraag aanpassen?

Het spijt ons dat het overzicht op blz. 12 voor verwarring heeft gezorgd. Deze afbeelding is aangepast. Om het aantal muizen in het project te verlagen en de haalbaarheid van het project te waarborgen wordt [redacted] alleen gedaan in [redacted] muizen. De experimenten met de [redacted] muizen zullen beperkt worden tot [redacted] zoals omschreven in DAP 3.4.4.2.

Kunt u de tekst onder kopje 'D. Replacement, reduction, refinement' in alle bijlages dierproeven verder uit te werken. De beschrijving van de toepassing van de methoden voor vervanging, vermindering en verfijning is summier. Vooral de methoden voor vervanging ontbreken in uw aanvraag.

De tekst onder 'Replacement, reduction, refinement' is in alle bijlagen verder uitgewerkt met de focus voornamelijk op informatievoorziening over vervanging.

Centrale Commissie Dierproeven

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Onze referentie
Aanvraagnummer
AVD103002016801
Bijlagen
1

Datum 1 februari 2017
Betreft Beslissing aanvraag projectvergunning Dierproeven

Geachte [redacted]

Op 22 december 2016 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Development of a cell therapy against the muscular phenotype of Myotonic Dystrophy " met aanvraagnummer AVD103002016801. Wij hebben uw aanvraag beoordeeld.

Op 16 januari 2017 heeft u uw aanvraag aangevuld. U heeft de vragen van de CCD beantwoord, het aantal dieren aangepast en de 3V's uitgewerkt.

Beslissing
Wij keuren uw aanvraag goed op grond van artikel 10a van de Wet op de Dierproeven (hierna: de wet). Hierbij gelden de voorwaarden zoals genoemd in de vergunning.
Met het oog op artikel 10a, lid 1, zijn er algemene voorwaarden gesteld.

U kunt met uw project "Development of a cell therapy against the muscular phenotype of Myotonic Dystrophy " starten. De vergunning wordt afgegeven van 1 februari 2017 tot en met 22 januari 2022. De looptijd van de vergunning wijkt af omdat de startdatum in de aanvraag in het verleden ligt.

Overige wettelijke bepalingen blijven van kracht.

Procedure
Bij uw aanvraag heeft u een advies van de Dierexperimentencommissie RU DEC gevoegd. Dit advies is opgesteld op 21 december 2016. Bij de beoordeling van uw aanvraag is dit advies betrokken overeenkomstig artikel 10a, lid 3 van de wet.
Wij kunnen ons vinden in de inhoud van het advies van de Dierexperimentencommissie. Dit advies van de commissie nemen wij over,

inclusief de daaraan ten grondslag liggende motivering. Er worden aanvullende algemene voorwaarde(n) gesteld.
Het DEC-advies en de in de bijlage opgenomen beschrijving van de artikelen van de wet- en regelgeving zijn de grondslag van dit besluit.

Bezwaar
Als u het niet eens bent met deze beslissing, kunt u binnen zes weken na verzending van deze brief schriftelijk een bezwaarschrift indienen.
Een bezwaarschrift kunt u sturen naar Centrale Commissie Dierproeven, afdeling Juridische Zaken, postbus 20401, 2500 EK Den Haag.

Bij het indienen van een bezwaarschrift vragen we u in ieder geval de datum van de beslissing waartegen u bezwaar maakt en het aanvraagnummer te vermelden. U vindt deze nummers in de rechter kantlijn in deze brief.

Bezwaar schorst niet de werking van het besluit waar u het niet mee eens bent. Dat betekent dat dat besluit wel in werking treedt en geldig is. U kunt tijdens deze procedure een voorlopige voorziening vragen bij de Voorzieningenrechter van de rechtbank in de woonplaats van de aanvrager. U moet dan wel kunnen aantonen dat er sprake is van een spoedeisend belang.

Voor de behandeling van een voorlopige voorziening is griffierecht verschuldigd. Op http://www.rechtspraak.nl/Organisatie/Rechtbanken/Pages/default.aspx kunt u zien onder welke rechtbank de vestigingsplaats van de aanvrager valt.

Meer informatie
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Centrale Commissie Dierproeven
namens deze:

[redacted]

ir. G. de Peuter
Algemeen Secretaris

Bijlagen:
- Vergunning
  Hiervan deel uitmakend:
  - DEC-advies
  - Weergave wet- en regelgeving

Centrale Commissie Dierproeven

Projectvergunning

gelet op artikel 10a van de Wet op de Dierproeven

Verleent de Centrale Commissie Dierproeven aan

Naam:	Stichting Katholieke Universiteit Nijmegen
Adres:	Postbus 9101, t.a.v. [redacted]
Postcode en plaats:	6500 HB NIJMEGEN
Deelnemersnummer:	10300

deze projectvergunning voor het tijdvak 1 februari 2017 tot en met 22 januari 2022, voor het project "Development of a cell therapy against the muscular phenotype of Myotonic Dystrophy " met aanvraagnummer AVD103002016801, volgens advies van Dierexperimentencommissie RU DEC. Er worden aanvullende algemene voorwaarde(n) gesteld.
De functie van de verantwoordelijk onderzoeker is Promovenda.
De aanvraag omvat de volgende bescheiden:

1 een aanvraagformulier projectvergunning dierproeven, ontvangen op 22 december 2016
2 de bij het aanvraagformulier behorende bijlagen:
a Projectvoorstel, zoals ontvangen per digitale indiening op 16 januari 2017;
b Niet-technische Samenvatting van het project, zoals ontvangen per digitale indiening op 16 januari 2017;
c Advies van dierexperimentencommissie d.d. 21 december 2016, ontvangen op 22 december 2016.
d De aanvullingen op uw aanvraag, ontvangen op 16 januari 2017

Naam proef	Diersoort/ Stam	Aantal dieren	Ernst	Opmerkingen
3.4.4.1 [redacted] skeletal muscle				
	Muizen (Mus musculus) / [redacted]	455	71% Matig 29% Licht	
3.4.4.2 [redacted] or saline solution				
	Muizen (Mus musculus) / [redacted]	152	60% Matig 40% Licht	
3.4.4.3 [redacted]				
	Muizen (Mus musculus) / [redacted]	43	100% Matig	

Aanvraagnummer:
AVD103002016801

Voorwaarden
Op grond van artikel 10a1 lid 2 van de Wet op de dierproeven zijn aan een projectvergunning voorwaarden te stellen

De vergunning wordt verleend onder de voorwaarde dat go/no go momenten worden afgestemd met de IvD.

In artikel 10, lid 1 sub a van de wet, wordt bepaald dat het verboden is een dierproef te verrichten voor een doel dat, naar de algemeen kenbare, onder deskundigen heersende opvatting, ook kan worden bereikt anders dan door middel van een dierproef, of door middel van een dierproef waarbij minder dieren kunnen worden gebruikt of minder ongerief wordt berokkend dan bij de in het geding zijnde proef het geval is. Nieuwe onderzoeken naar alternatieven kunnen tot gevolg hebben dat inzichten en/of omstandigheden van het aangevraagde project in de vergunningsperiode wijzigen, gedurende de looptijd van deze vergunning. Indien bovenstaande zich voordoet dient aanvrager dit in afstemming met de IvD te melden bij de CCD. De CCD kan in een dergelijke situatie aan de vergunning nieuwe voorwaarden verbinden en gestelde voorwaarde wijzigen of intrekken.

Aanvraagnummer:
AVD103002016801

Weergave wet- en regelgeving

Dit project en wijzigingen
Volgens artikel 10c van de Wet op de Dierproeven (hierna de wet) is het verboden om andere dierproeven uit te voeren dan waar de vergunning voor is verleend. De dierproeven mogen slechts worden verricht in het kader van een project, volgens artikel 10g. Uit artikel 10b volgt dat de dierproeven zijn ingedeeld in de categorieën terminaal, licht, matig of ernstig. Als er wijzigingen in een dierproef plaatsvinden, moeten deze gemeld worden aan de Centrale Commissie Dierproeven. Hebben de wijzigingen negatieve gevolgen voor het dierenwelzijn, dan moet volgens artikel 10a5 de wijziging eerst voorgelegd worden en mag deze pas doorgevoerd worden na goedkeuren door de Centrale Commissie Dierproeven.
Artikel 10b schrijft voor dat het verboden is een dierproef te verrichten die leidt tot ernstige mate van pijn, lijden, angst of blijvende schade die waarschijnlijk langdurig zal zijn en niet kan worden verzacht, tenzij hiervoor door de Minister een ontheffing is verleend.

Verzorging
De fokker, leverancier en gebruiker moeten volgens artikel 13f van de wet over voldoende personeel beschikken en ervoor zorgen dat de dieren behoorlijk worden verzorgd, behandeld en gehuisvest. Er moeten ook personen zijn die toezicht houden op het welzijn en de verzorging van de dieren in de inrichting, personeel dat met de dieren omgaat moet toegang hebben tot informatie over de in de inrichting gehuisveste soorten en personeel moet voldoende geschoold en bekwaam zijn. Ook moeten er personen zijn die een eind kunnen maken aan onnodige pijn, lijden, angst of blijvende schade die tijdens een dierproef bij een dier wordt veroorzaakt. Daarnaast zijn er personen die zorgen dat een project volgens deze vergunning wordt uitgevoerd en als dat niet mogelijk is zorgen dat er passende maatregelen worden getroffen.
In artikel 9 staat dat de persoon die het project en de dierproef opzet deskundig en bekwaam moet zijn. In artikel 8 van het Dierproevenbesluit 2014 staat dat personen die dierproeven verrichten, de dieren verzorgen of de dieren doden, hiervoor een opleiding moeten hebben afgerond.
Voordat een dierproef die onderdeel uitmaakt van dit project start, moet volgens artikel 10a3 van de wet de uitvoering afgestemd worden met de instantie voor dierenwelzijn.

Pijnbestrijding en verdoving
In artikel 13 van de wet staat dat een dierproef onder algehele of plaatselijke verdoving wordt uitgevoerd tenzij dat niet mogelijk is, dan wel bij het verrichten van een dierproef worden pijnstillers toegediend of andere goede methoden gebruikt die de pijn, het lijden, de angst of de blijvende schade bij het dier tot een minimum beperken. Een dierproef die bij het dier gepaard gaat met zwaar letsel dat hevige pijn kan veroorzaken, wordt niet zonder verdoving uitgevoerd. Hierbij wordt afgewogen of het toedienen van verdoving voor het dier traumatischer is dan de dierproef zelf en het toedienen van verdoving onverenigbaar is met het doel van de dierproef. Bij een dier wordt geen stof toegediend waardoor het dier niet meer of slechts in verminderde mate in staat is pijn te tonen, wanneer het dier niet tegelijkertijd voldoende verdoving of pijnstilling krijgt toegediend, tenzij wetenschappelijk gemotiveerd. Dieren die pijn

kunnen lijden als de verdoving eenmaal is uitgewerkt, moeten preventief en postoperatief behandeld worden met pijnstillers of andere geschikte pijnbestrijdingsmethoden, mits die verenigbaar zijn met het doel van de dierproef. Zodra het doel van de dierproef is bereikt, moeten passende maatregelen worden genomen om het lijden van het dier tot een minimum te beperken.

Einde van een dierproef
Artikel 13a van de wet bepaalt dat een dierproef is afgelopen wanneer voor die dierproef geen verdere waarnemingen hoeven te worden verricht of, voor wat betreft nieuwe genetisch gemodificeerde dierenlijnen, wanneer bij de nakomelingen niet evenveel of meer, pijn, lijden, angst, of blijvende schade wordt waargenomen of verwacht dan bij het inbrengen van een naald. Er wordt dan door een dierenarts of een andere ter zake deskundige beslist of het dier in leven zal worden gehouden. Een dier wordt gedood als aannemelijk is dat het een matige of ernstige vorm van pijn, lijden, angst of blijven schade zal blijven ondervinden. Als een dier in leven wordt gehouden, krijgt het de verzorging en huisvesting die past bij zijn gezondheidstoestand.
Volgens artikel 13b moet de dood als eindpunt van een dierproef zoveel mogelijk worden vermeden en vervangen door in een vroege fase vaststelbare, humane eindpunten. Als de dood als eindpunt onvermijdelijk is, moeten er zo weinig mogelijk dieren sterven en het lijden zo veel mogelijk beperkt blijven.
Uit artikel 13d volgt dat het doden van dieren door een deskundig persoon moet worden gedaan, wat zo min mogelijk pijn, lijden en angst met zich meebrengt. De methode om te doden is vastgesteld in de Europese richtlijn artikel 6.
In artikel 13c is vastgesteld dat proefdieren geadopteerd kunnen worden, teruggeplaatst in hun habitat of in een geschikt dierhouderijsysteem, als de gezondheidstoestand van het dier het toelaat, er geen gevaar is voor volksgezondheid, diergezondheid of milieu en er passende maatregelen zijn genomen om het welzijn van het dier te waarborgen.
De Minister heeft vrijstelling ontheffing verleend volgens artikel 13c, die de afwijkende methode van doden op basis van wetenschappelijke motivering ten minste even humaan acht als de in de richtlijn opgenomen passende methoden.