	Inventaris Wob-verzoek W16-01								
		wordt verstrekt				**weigeringsgronden**			
nr.	**document**	**reeds openbaar**	**niet**	**geheel**	**deels**	**10.1.c**	**10.2.e**	**10.2.g**	**11.1**
	NTS 20151								
1	Aanvraagformulier				x		x	x	
2	Niet-technische samenvatting	x							
3	Projectvoorstel				x			x	
4	Bijlage beschrijving dierproeven 1			x					
5	Bijlage beschrijving dierproeven 2			x					
6	Bijlage beschrijving dierproeven 3			x					
7	Bijlage beschrijving dierproeven 4			x					
8	Bijlage beschrijving dierproeven 5			x					
9	Appendix			x					
10	DEC-advies				x		x	x	
11	Ontvangstbevestiging				x		x	x	
12	Verzoek aanvullende informatie				x		x	x	
13	Aanvullende informatie				x		x	x	
14	Verzoek aanvullende informatie DEC				x		x	x	
15	Aanvullende informatie DEC				x		x	x	
16	Advies CCD		x						x
17	Beschikking en vergunning				x		x	x	

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* 80102 (Hubrecht Instituut-KNAW)
☐ Nee > *U kunt geen aanvraag doen*

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

Naam instelling of organisatie	KNAW
Naam van de portefeuillehouder of diens gemachtigde	[redacted]
KvK-nummer	5 4 6 6 7 0 8 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	
Postbus	Postbus 19121
Postcode en plaats	1000GC Amsterdam
IBAN	NL94DEUT0552026182
Tenaamstelling van het rekeningnummer	Hubrecht Instituut

1.4 Vul de gegevens in van de verantwoordelijke onderzoeker.

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

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

(Titel) Naam en voorletters	[redacted] ☑ Dhr. ☐ Mw.
Functie	Senior Scientist
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	☐ Dhr. ☐ Mw.
		Functie	
		Afdeling	
		Telefoonnummer	
		E-mailadres	
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	01-03-2015
		Einddatum	01-03-2020
3.2	Wat is de titel van het project?	The role of stem cells and their derivatives during development, adult tissue homeostasis	
3.3	Wat is de titel van de niet-technische samenvatting?	Adulte stamcellen: van identificatie tot regeneratieve geneeskunde.	
3.4	Wat is de naam van de Dierexperimentencommissie (DEC) aan wie de instellingsvergunninghouder doorgaans haar projecten ter toetsing voorlegt?	Naam DEC	DEC-KNAW
		Postadres	[redacted] Amsterdam
		E-mailadres	[redacted]

4 Betaalgegevens

4.1 Om welk type aanvraag gaat het?

- [x] Nieuwe aanvraag Projectvergunning € 741,00 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.

- [x] Via een eenmalige incasso
- [ ] Na ontvangst van de factuur

5 Checklist bijlagen

5.1 Welke bijlagen stuurt u mee?

Verplicht

- [x] Projectvoorstel
- [x] Niet-technische samenvatting

Overige bijlagen, indien van toepassing

- [ ] Melding Machtiging
- [x] Flow chart experimenten

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	[redacted]
Plaats	Amsterdam
Datum	11-02-2015
Handtekening	[redacted]

Form
Project proposal

- This form should be used to write the project proposal for 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'.	80102 (Hubrecht Instituut)
1.2	Provide the name of the licenced establishment.	KNAW-Hubrecht Instituut-Developmental Biology and Stem cells
1.3	Provide the title of the project.	The role of stem cells and their derivatives during development, adult tissue homeostasis, diseases and regenerative medicine

2 Categories

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

- ☒ Basic research
- ☐ Translational or applied research
- ☐ Regulatory use or routine production
- ☐ Research into environmental protection in the interest of human or animal health or welfare
- ☐ 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.

Stem cells are the linchpins of tissue renewal, repair and remodelling during development and in mature organs. Dysfunction in these processes is a driving cause of many diseases (e.g. cancer, degenerative and immune disorders, organ failure and diabetes). Concurrently, adult stem cells hold great promise for regenerative medicine strategies. Recent years have witnessed a tremendous increase in research into basic and clinical aspects of stem cells. However, approaches to regenerate or specifically treat tissues in diseased or damaged organs have often been hampered by the lack of detailed knowledge on the biology of the adult stem cells of the pertinent organ. This knowledge requires the identification, isolation and in depth characterization of stem cells and the identification and understanding of the processes that control the behaviour of stem cells and their derivatives under normal and pathological conditions. Indeed, our solid fundamental research of among others the intestinal stem cells strongly increased our current knowledge of stem cells and showed the importance and feasibility of this research for effective treatment and transplantation. Although some general rules apply across different tissues, each tissue appears to employ uniquely designed stem cell hierarchies and, correspondingly, unique tissue architectures to fulfil specific physiologic demands. Therefore, to gain insight in tissue homeostasis and regeneration in other organs, the identification and characterization of (additional) tissue specific quiescent and proliferating stem cell specific markers and their derivatives for the different (developing) healthy and diseased organs are of utmost importance. This fundamental knowledge may ultimately open new avenues for a variety of new and/or improved therapeutic strategies.

In mammals, embryonic stem (ES) cells and adult stem cells comprise two broad categories of stem cells. ES cells, present in the inner cell mass of early embryos (blastocysts), are pluripotent stem cells characterized by their ability to differentiate into derivatives of all three germ layers: ectoderm, endoderm, and mesoderm. These derivatives include each of the more than 200 cell types in the adult body. As development proceeds, ES cells gradually lose their plasticity, ultimately giving rise to adult tissues each harbouring a limited reservoir of tissue-specific adult stem cells. Adult stem cells maintain themselves long-term as a population (self-renewal), whilst also supplying all differentiated cell types of the pertinent tissue (multi-potency).

To be able to perform their essential functions, adult stem cells are subjected to tight regulatory processes so that they are activated and give rise to the right number and type of differentiated progeny at the appropriate time and place in a given biological setting. In that regard, stem cells must also be mobilized quickly in response to injury (regeneration), but once the tissue has been restored, stem cells must also be able to revert back to their 'normal' state. The ability of stem cells to sense and respond to tissue needs is controlled by their interplay between intrinsic transcriptional programs and their intimate association with stem cell niches. Stem cell niches are specific anatomic locations, present in every organ, which are composed of micro environmental cells that nurture epithelial stem cells and enable them to maintain tissue homeostasis.

Our fundamental research, especially about the intestine, has made great strides of our knowledge of these processes. The epithelium of the intestine is exposed to continuous mechanical wear-and-tear and, as a consequence, has to be renewed every week. In this highly dynamic organ we have identified and fully characterized the stem cell, their niche, their transcriptional programs, their role in tissue homeostasis and regeneration and their role as initiating cell of

intestinal tumors (e.g. 1-9). Epithelia of other organs, such as liver, pancreas or kidney, are self-renewing at a low rate or are in a "quiescent/dormant" state when compared to the intestine (10-12). These quiescent epithelial stem cells are activated during the regenerative response following damage. Although general rules do apply across these different organs, each organ appears to employ uniquely designed stem cell hierarchies and, correspondingly, unique tissue architectures to fulfil specific physiologic demands. Therefore, to gain insight in tissue homeostasis and regeneration in the different organs, the identification and characterization of (additional) tissue specific quiescent and/or proliferating stem cells, their derivatives and their niches for the different (developing) organs are of utmost importance.

The activation of aberrant molecular signalling pathways in adult stem cells and/or their niche resulting in dysfunctional behaviour has been associated with diseases. E.g. the accumulation of gene alterations occurring specifically in adult epithelial stem cells could trigger their transformation into malignant cells which contributes to their sustained growth, survival, expansion and metastasis (8,13). Insight into stem cells and their niches in terms of their function in pathophysiological condition is therefore of fundamental importance to understand their normal biology and to understand their role in malignancy. This knowledge will, ultimately, contribute to develop effective strategies to treat diseases.

Over the years, our extensive genetic studies in mice have provided insights into growth factor–dependency of intestinal epithelial stem cells. This knowledge was the basis of the establishment of a unique long-term in vitro culture model in which 3-dimensional (3D), karyotype stable, intestinal organoids derived from a single intestinal epithelial stem cell can be grown indefinitely (14). The architecture of these three-dimensional organotypic structures is remarkably similar to that of the normal intestinal epithelium. Moreover, the organoids recapitulate the epithelial stem cell-differentiation hierarchy and allow in vitro studies of cell fate determination. To date, we established organotypic ex vivo culture systems from multiple human and murine epithelial organs, including the liver, pancreas, stomach, small intestine, prostate, salivary glands and colon (15-18). We are currently trying to find c.q. optimize growth conditions for culturing organoids derived from other organs. We also applied this culture system to diseased epithelia, such as intestinal adenoma, adenocarcinoma and Barrett's epithelium (19,20). Since organoids are amenable to all standard experimental manipulations used for cell lines this technique allows us, among others, to study gene function in murine epithelial cells ex vivo. Indeed, we are able to create near-physiological ex vivo models of human diseases. Therefore, genetic manipulation of organoids is currently our successful alternative method for the generation and analysis of genetically modified mice (GGM). However, the generation of novel GGM is sometimes still required to study the full effect of a gene alteration in context with all molecular, developmental and physiological interactions provided in vivo within an intact entire mammalian organ.

We have successfully employed novel genome-editing techniques to functionally repair disease loci in stem cells of intestinal organoids from human cystic fibrosis patients (21). In addition, we have successfully transplanted organoids derived from their respective stem cells in murine colon, pancreas and liver tissue. It is of fundamental importance to further understand and optimize all the transplantation requirements in the aforementioned and other organs. It is anticipated that these (combined) technologies will be instrumental in exploiting the possibilities of the usage of (genetically modified) organoids in regenerative medicine and/or treating human disorders.

In conclusion, in this multi-organ project we will build on the many observations we have made using patients material, existing datasets and in vitro and in vivo murine models to further refine our understanding of the role of stem cells, their niches and their derivatives in (developmental) biology and disease and for the development of therapies influencing or using stem cells and/or organoids to modulate healing.

To obtain answers to these important fundamental questions, a wide variety of experiments including the generation, treatment and careful analysis of novel and existing genetically modified (compound) mouse models is required.

1) Barker, N, van Es, J.H., Kuipers, J., Kujala P., van den Born, M., Cozijnsen, M., Korving, J., Begthel, H., Peters, P.C., and Clevers, H. Identification of Stem Cells in Small Intestine and Colon by a Marker Gene LGR5. Nature, 449:1003-1007 (2007)
Keywords: intestine, identification stem cells, lineage tracing.
It was obvious that the intestine should have a stem cell population which was responsible for the replacement of the enormous amount of differentiated cells lost on a daily basis. However, the location of the intestinal stem cell was was heavily debated. In this study we have identified and marked the genuine intestinal stem cell.

2) Van Es J.H., et al. Nature 435:959-963 (2005)
3) Haramis A.P., et al. Science. 303:1684-6 (2004)
4) Korinek, V., et al. Nat Genet 19(4):379-383 (1998)
5) Battle, E., et al.. Cell 111:251-263 (2002)
6) Sato, T., van Es, J.H., Snippert, H.J., Stange, D.E., Vries, R.G., van den Born, M., Barker, N., Shroyer, N.F., van de Wetering, M., Clevers, H. Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. Nature 469:415-418 (2010). Keywords: intestine, tissue homeostasis, niche.
It was unknown how Lgr5+ intestinal stem cells maintain their stemness. It has been shown that cells in the surrounding mesenchyme produce important (growth) factors. In this study we described the role of Paneth cells, one of the 5 major differentiated cells of the intestine, as an essential niche cell of the stem cells. They are performing this function via the production of essential growth factors and giving the correct signals via certain membrane receptors.
7) van Es, J.H., et al. Nat Cell Biol 14:1099-1104 (2012)
8) Barker N., Ridgway R.A., van Es J.H.,van de Wetering M., Begthel H., van den Born M., Danenberg E., Clarke A.R., Sansom O.J., Clevers H. Crypt Stem Cells as the Cells-of-Origin of Intestinal Cancer. Nature 457:608-611(2009). Keywords: intestine, stem cells, deregulation signalling pathway, cancer.
Intestinal cancer is initiated by Wnt-pathway-activating mutations in genes such as adenomatous polyposis coli (APC). As in most cancers, the cell of origin has remained elusive. In this study we unequivocally demonstated that the Lgr5+ stem-cell is the cell of origin for cancer upon specific loss of Apc resulting in progressively growing neoplasia.
9) van der Flier, L.G., et al. Cell 136:903-12 (2009)
10) Huch M., Dorell, C., Boj, S.F., van Es, J.H., van de Wetering, M., Li, V.S.W., Hamer, K., Sasaki, N., Finegold, M.J., Haft, A., Grompe, M., Clevers, H. In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration. Nature 494:247-250 (2013). Keywords: liver, stem cells, lineage tracing, injury, organoids, Wnt signaling pathway, transplantation.
In this study we described Lgr5 as a marker for stem cells of the liver. However, in contrast to the Lgr5 stem cell of the intestine, the lgr5 liver stem cell is only activated upon damage of the liver. Moreover, we described a three-dimensional culture system for the liver (so-called liver organoids). In addition, we were able to transplant these liver organoids in mice with a diseased liver. This study opened up many exciting translational opportunities in the biomedical sectors (e.g. the treatment of patients with certain liver diseases via the transplantation of healthy organoids) and in basic research into epithelial biology (e.g as a replacement for some in vivo studies).
11) Barker N, et al. Cell Rep. 2(3):540-52 (2012)
12) Stange, D.E., et al. Cell 155:357-368 (2013). keywords: stomach, tracing, tissue damage, organoids
In this study we showed the presence of a unique type of stem cell in the stomach. This cell is actually a subset of a differentiated cell (so-called Chief cell) which is able to revert into stem cell only upon damage of the tissue. This study shows a unique mechanism how an organ is able to recover from tissue damage via activation of quiescent stem cells.
13) Batlle E., et al. Nature 435:1126-1130 (2005)
14) Sato, T., et al. Nature 459 :262-5 (2009). Keywords: intestine, stem cells, organoids, in vitro tracing.
In this study we described the establishment of a unique long-term in vitro culture model in which 3D, intestinal organoids can be grown indefinitely. This development opened up many exciting translational opportunities in the biomedical sectors (e.g. the treatment of human gastrointestinal diseases (e.g. MIVD) via the transplantation of healthy organoids) and in basic research into epithelial biology (e.g as a replacement for some in vivo studies).
15) Huch M, et al. EMBO J. 32(20):2708-219 (2013)
16) Barker, N, et al. Cell Stem Cell, 6:25-36 (2010)
17) Karthaus WR, et al. Cell 159:163-75 (2014)
18) Huch M, et al. Cell 160(1-2):299-312 (2015)
19) Sato T, et al. Gastroenterology. 2011 141(5):1762-72 (2011)
20) Boj SF, et al. Cell. 160(1-2):324-38. (2015)

21) Schwank G, Koo BK, Sasselli V, Dekkers JF, Heo I, Demircan T, Sasaki N, Boymans S, Cuppen E, van der Ent CK, Nieuwenhuis EE, Beekman JM and Clevers H. Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. Cell Stem Cell 13:653-658 (2013). Keywords: organoids, disease (CFTR), gene correction.
In this study we showed that we are able to correct a mutated gene (CFTR: the mutated gene causes cystic fibrosis) via a novel genome editing system in our unique organoid culture system. This might open the road to treat certain patients via the transplantation of gene corrected organoids.

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?

Our aim is to define and understand the intrinsic and extrinsic signal of stem cells and their derivatives in the different tissues that regulate embryonic development and/or adult tissue homeostasis, their contribution to the genesis of (malignant) disorders and for the usage of stem cell derived organoids for regenerative medicine.
More specifically, the key questions we want to address are:
1) What is the role of stem cells and their derivatives during development and in tissue homeostasis? (Protocol 1-3)
2) What is the role of stem cells and their derivatives in regeneration upon (induced) injury? (Protocol 1-3)
3) What is the role of stem cells, their derivatives and their niches during pathogenesis and how can we specifically manipulate, prevent or alleviate these cells c.q. interactions? (Protocol 1-4)
4) Can we find c.q. optimize growth conditions for culturing organoids derived from organs of genetically modified (compound) and/or wild type murine mice? (Protocol 2, 4)
5) Can we use the wild type and/or functionally repaired and/or genetically modified organoids as a tool for their usage in regenerative medicine and/or disease models? (Protocol 2-5)

There are several reasons why we think that we will achieve our aims.

We are embedded in the lively scientific environment of the Hubrecht Institute in Utrecht. The research at the Hubrecht Institute focuses on developmental biology and stem cells at the organismal, cellular, genetic, genomic and proteomic level. The aim is to gain insight into development and stem cells and their relation to (human) disease, such as cancer. The Hubrecht Institute provide core facilities for deep sequencing, transgenesis, histology, fluorescent imaging by confocal and 2-photon technology, mRNA expression arraying, FACS-based cell sorting etc. The dedicated staff at the animal unit will provide the regular housing of the animals. The dedicated scientist and technicians performing the mouse experiments are very well trained and experienced. We have very strong collaborations with national and international groups. [redacted] consists of highly motivated and skilled senior scientists (3), PostDocs (12), PhD students (5), and technicians (8). Our research is regularly evaluated within our group and institute meetings and has been positively judged by many different financing organizations including KWF, EC, BSIK, NWO, NIH en ZONMW. These organizations use independent external reviewers. Their positive judgments show that our research is of major scientific significance and of the highest quality. Moreover, the quality of our work is further underscored by the many (inter)national prizes [redacted] received for our work and the many peer reviewed publications in the most highly respected scientific journals ((e.g. Nature (n=15), Science (n=5), Cell (n=12), Developmental Cell, Nature Cell Biology (n=2), Nature Genetics (n=2), Nature Communication, Nature Protocols, Nature Methods, Cell Stem Cell (2 times), Cell report, Stem Cell Reports (n=2)) and the many invited reviews in the most highly respected scientific journals and books (e.g. Cell (n=8), Science (n=5), Cell Stem Cell (n=2), Genes and Development (n=3), Cancer Cell, Nature Rev. Immun., Nature Rev. Drug Disc., Nature Rev. Cancer, Nature Med, Nature Cell Biology, Nature Biotechnology, Nature (n=2), Immunity, Annu Rev Physiol., Yamada's textbook for Gastroenterology). The combined factors make it very likely that we, as in the past, will achieve our aims.

3.3 Relevance

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

This work is expected to provide novel scientific knowledge about the role of stem cells and their derivatives in various (developing) organs; (1) the pathways that regulate their maintenance and differentiation in normal tissue homeostasis and during regeneration, (2) the interaction of stem cells with their niche cells, and (3) the changes in niche and/or stem cells that result in disorders. Moreover, the requirements for the usage of (genetically modified) organoids for transplantation purposes in mouse model system(s) will be investigated.

This fundamental knowledge might, upon proof of principle in mouse model system(s), ultimately facilitate the development of novel and/or improved human therapies designed to achieve tissue regeneration and for the treatment of diseases for many internal organs as have been shown before with e.g. haematopoietic stem cell, skin and hair transplantation.

Moreover, our organoid in vitro culture system is for many experiments already a good alternative for in vivo studies, is used in toxicology studies and is used for the generation of patient and/or disease specific in vitro model for (personalized) drug screening and drug development for several diseases.

3.4 Research strategy

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

Each organ, although general rules apply across the different organs, employs uniquely designed stem cell hierarchies and, correspondingly, unique tissue architectures to fulfill specific physiologic demands. Indeed, the epithelium of the human intestine is exposed to mechanical wear-and-tear and as a consequence has to produce 100.000.000.000 new intestinal cells every day! The epithelial stem cells of other organs, such as liver, pancreas, or kidneys, are self-renewing at a much lower rate or are even in a "quiescent/dormant" state. These quiescent epithelial stem cells are only activated during the regenerative response following damage. Our aim is to define and understand of all organs the intrinsic and extrinsic signal of epithelial stem cells and their derivatives in the different organs that regulate embryonic development and/or adult tissue homeostasis and contribute to the genesis of (malignant) disorders and for the usage of organoids for regenerative medicine.

See Flow Chart in attachment 1.

Based on published data in the literature and/or gene expression profiles derived from different isolated (stem cell) populations from the different organs, organoids, patient material or cell lines (putative) interesting genes will be selected. The identified gene(s) will initially be carefully tested on patient material, organoids and/or cell lines. Only if the identified genes show an interesting phenotype in these in vitro experiments, we will consider the extensive and careful analysis of (compound) GM mice. If so, we will import the relevant existing mouse line or, if required, we will generate the new mouse line(s). The initial in vitro experiments reduce the number of animals (3Rs).

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.

See also Flow Chart in attachment 1.

1) Generation of GM Mice (described in detail in 3.4.4.1)

If we have identified a putative important gene, we will import the relevant existing mouse line or, if required, we will generate the new mouse line via standard oocyte injection, blastocyst injection or via the Crispr/Cas9 system. The Crispr/Cas9 system will especially be used as a highly efficient tool for simultaneously multi-gene editing. This prevents the generation and breeding of multiple homozygotes from individually targeted ES cells (3R's).

2) Welfare assessment for 2 generations in novel (compound) mouse models (described in detail in 3.4.4.1).

We will identify a possible hampered phenotype in novel (compound) mouse models according to the guidelines of the new EU directive. Therefore new transgenic lines and/or KO lines generated via classical methods and/or novel combinations of these aforementioned lines will be monitored for 2 generations to determine the absence or presence of discomfort. We will daily check the mice on several parameters (overall appearance, size, growth, coat condition, behavior, clinical signs, relative size and numbers) as has been described in the Directive 2010/63/EU: corrigendum of 24 Jan. 2013. We will immediately kill the mice a hampered phenotype will show up.

3) Tissue, lineage and/or cell type specific gene (in)activation and (over)/(mis)expression (described in detail in 3.4.4.2).

Once candidate gene(s) have been obtained, we might elucidate the function of these genes in vivo, during development and/or tissue homeostasis. This will be carried out via analysis of GM mice in which the gene(s) will be activated, inactivated, overexpressed and/or misexpressed.

In contrast to the conventional gene-targeting strategy, the use of e.g. the Cre/LoxP recombination system in conjunction with gene targeting has greatly expanded the versatility and avenues with which biologic questions can be addressed in the mouse. This system allows us, by strategically incorporating Cre recombinase recognition (LoxP) sites into the genome and the subsequent expression of the Cre recombinase, to study the consequence of specific ablation, activation and/or over/misexpression of a specific protein. In particular, when Cre is expressed in mice harboring a LoxP-containing target gene, the desired gene modification can be restricted to certain developmental stage, organ (e.g. intestine), cell type (e.g. stem cell), pathogenesis (e.g. cancer cells) of the mouse depending on the specificity and timing of recombinase expression.

Moreover, the introduction of novel gene(s) will help us in further characterizing the role of expressing cells. E.g. the introduction of e.g. a fluorescent marker in a (putative) stem cell allows us to isolate these cells via FACS sorting upon which we could e.g. determine the gene expression profile. Moreover, the (combined) introduction of e.g. an exogenous toxin receptor in the same cells allows us to specifically kill these cells upon the administration of the toxin to the mouse, allowing us to determine the consequence of this cell depletion during development and/or in tissue homeostasis. This is also true for over or mis- or overexpression of (mutated) genes.

Finally, by administering small molecule compounds/drugs/toxin/chemical or control substances (e.g. inhibitors or agonists of specific pathways), we might be able to rescue or mimic the phenotypes of the in vivo genetic deletions and/or activations and therefore further identify the function of these cells in vivo. If possible and/or relevant, we will always test these small molecule compounds/drugs/chemicals first on in vitro growing organoids and in case relevant effects are observed shift to in the in vivo models.

In the overwhelming majority of our mice experiments in which we specifically (in)activate and/or (mis)/(over)express genes in a tissue, lineage or cell type specific manner, we are only interested in the analysis of phenotypic effects at an early stage of the genetic modification, which means before discomfort might develop.

4) Lineage tracings studies (described in detail in 3.4.4.3).

In lineage tracing, a single cell is marked in such a way that the mark is transmitted to the cell's progeny, resulting in a set of labelled clones. Lineage tracing provides information about the number of progeny of the founder cell, their location and their differentiation status. Genetic lineage tracing in mice is nowadays usually performed using the well-known Cre-LoxP system. Creert2 recombinase is therefore expressed under the control of e.g. (stem)cell-specific promoter in one mouse line. That line is crossed with a second mouse line in which a reporter is flanked by a LoxP-STOP-LoxP sequence. In animals expressing both constructs, upon Cre induction via tamoxifen administration, the Cre enzyme specifically activates the reporter (e.g. LacZ or Fluorescent marker) in cells that express the promoter, by permanently excising the STOP sequence in these cells and their progeny. Indeed, the identification and specific dynamics of the Lgr5 expressing stem cell during development, in normal homeostasis and diseases (cancer) in e.g. the intestine has been successfully investigated using this in vivo lineage-tracing approach. The obtained data unequivocally demonstrated that e.g. Lgr5 marks the genuine intestinal stem cell. Moreover, Lgr5 expressing stem cells are the cell of origin for cancer upon (in)activation of certain (proto) oncogene(s). In other organs

(e.g. the pancreas), activation of the stem cell (and thereby lineage tracing) can only be induced upon an inflicted injury.

5) Regeneration (described in detail in 3.4.4.3)
To identify the role of (putative) stem cells during regeneration upon inflicted injury the following approach(es) will be undertaken.
To identify active and/or quiescent stem cell(s) in vivo, we will perform subsequently lineage tracing studies upon damage (via e.g. small molecule compounds, drugs, toxin, chemicals or control substances, irradiation, partial ectomy, ischemie and reperfusion, and/or genetic alterations). Novel or existing (stem) cell specific Cre lines will therefore be crossed with novel or existing inducible reporter mice (e.g. Fluorescent or LacZ).
To be able to isolate the cells responsible for regeneration, we also might earmark these cells by making Knock In mice with e.g. a fluorescent marker. This allows us to isolate these cells via FACS sorting followed via careful analysis via among others gene expression profiling and to follow regeneration in vivo via imaging.
To increase tissue regeneration response, validated chemicals, drugs and/or protein(s) (e.g. R-spondin) might be administrated.

6) Disease models caused by deregulated stem cells (mainly cancer models in different tissues)(described in detail in 3.4.4.4)
To identify the role of stem cells, their derivatives and their niches during pathogenesis and how to manipulate these cells/interactions the following approaches will be undertaken.
Once mice with candidate gene(s) have been obtained, we will elucidate the function of these cells and factors in vivo, during pathogenesis. For that, animals carrying e.g. Cre inducible alleles will be crossed with animals carrying floxed alleles in specific genes to delete and/or overexpress and/or misexpress these genes in vivo, following administration of the inducible drug (e.g. Tamoxifen for Cre induction).
To assess the function of these cells in pathogenesis in vivo, we might delete these cells by using cytotoxic systems, as the DTR/DTA (diphtheria toxin receptor/diphtheria toxin) or others, where the cytotoxic gene is (inducible) expressed specifically cell type (e.g. cancer stem cells). In the case of DTR expressing cells, the administration of diphtheria toxin will induce the cell death of the DTR expressing cell.
Finally, by administering small molecule compounds (e.g. inhibitors or agonists of specific pathways), drugs, toxins, chemicals or control substances we might be able to prevent, rescue or mimic the phenotypes of the in vivo genetic deletions and therefore identify the function of these cells during pathogenesis in vivo. If possible and/or relevant, we will always test these small molecule compounds on in vitro growing organoids and in the case relevant effects are observed shift to the in vivo models.
In the overwhelming majority of our mice experiments with disease models, we are only interested in the analysis of early stages of the disease, before any discomfort develop.

7) Growth and manipulation of organoids. (described in detail in 3.4.4.5).
We established organotypic ex vivo culture systems from multiple human and murine epithelial organs, including the liver, pancreas, stomach, small intestine, prostate, salivary glands and colon. This technology allows us to indefinitely grow so-called mini organs or organoids. Although, we are able to grow organoids from several tissues, we are still interested in optimizing their growth conditions and also finding the optimal growth conditions for other (diseased) organs such as lung, ovary etc. Moreover, we are interested in the generation, careful analysis and (additional) genetic modification of organoids generated from different organs derived from wild type and existing and novel (compound) genetically modified embryonic, neonatal and adult mice to further understand their role in development, tissue homeostasis and/or for pathogenesis.

8) (Xeno)transplantation of organoids (described in detail in 3.4.4.5).
We have successfully transplanted normal, in vitro and in vivo genetically modified murine organoids derived from individual stem cells in murine colon, pancreas and liver tissue.
Moreover, we are able to genetically manipulate organoids. This establishes the organoid system as a promising tool for adult stem cell-based gene

therapy. We are interested to use these (genetically modified) organoids for transplantation in wild type, injured and/or diseased organs. If possible transplantation conditions will first be validated in wild type animals before we will perform organoid transplantation in GG mice with injured and/or diseased organs.

Moreover, to assess whether the mutation of the selected genes on the quiescent and/or activated (stem) cell populations are relevant to human cancer, human (cancer) organoids or human organoids engineered to expressed the mutant genes will be engrafted in immunocompromised mice.

In summary, the described (combined) experiments on the role of stem cells and their derivatives during development, adult tissue homeostasis, diseases and regenerative medicine will strongly increase our fundamental insight in these processes and might show the importance and feasibility of this research for effective treatment and transplantation.

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.

See Flow Chart in attachment 1.

All experiments start with the identification of candidate genes. The identified gene(s) will initially be carefully tested on patient material, organoids and/or cell lines. Only if the identified genes showed an interesting phenotype in these in vitro experiments, we will consider the extensive and careful analysis of (compound) GM mice. The in vivo experiments might contain some or all of the following studies which will be carried out sequentially: Tissue, lineage and/or cell type specific gene (in)activation and (over)expression, lineage tracings studies i.a. organs with inflicted damage, regeneration, generation and analysis (i.a.) of disease models, the growth and manipulation of organoids, (xeno)transplantation of organoids. At every stage of every experiment we have go no go decisions. The decisions points for every experiment will be extensively described in our experimental protocols.

This multi-organ approach consists of similar, but due to the fact that different organs contain different (stem) cells with different characteristics, for the specific organ adapted approaches.

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	Generation of novel (compound) GM mice via state of the art technology and identification of phenotypes with constitional discomfort (Question 1-3,5*).
2	Tissue, lineage and/or cell type specific gene (in)activation and (over)/(mis)expression. (Question 1-3*).
3	Lineage tracings and regeneration (Question 1-3*).
5	Generation and analysis of disease models caused by deregulated stem cells (Question 1-3*).
6	Growth and genetically modification of organoids and (xeno)transplantation of (genetically manipulated) organoids in diseased, damaged or wild type organs (Question 1-5*).
7	
8	
9	
10	*) The question numbers refer to the questions described under 3.1.

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'.	80102
1.2	Provide the name of the licenced establishment.	KNAW-Hubrecht Institute Developmental Biology and Stem cells

1.3 List the serial number and type of animal procedure.

Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.

Serial number	Type of animal procedure
1	Generation of novel (compound) GM mice and identification of phenotype's with constitional discomfort.

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.

Creation of genetically mice via DNA/RNA injection into oocyte, injection of genetically modified ES cells into blastocysts and/or via the CRISPR/Cas9 system.

Welfare assessment for novel (compound) mouse models according to the guidelines of the new EU directive. New transgenic lines and/or KO lines generated

via classical methods and/or novel combinations of these aforementioned lines will be monitored for 2 generations to determine the absence or presence of mice with a hampered fenotype.

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

Generation:
1) Superovulation.
   a) Administration of gonadotropin's (2 times) by subcutaneous or intraperitoneal injections followed by mating.
   b) Animals will be killed for the isolation of early embryos.

2) Embryo recipients.
   a) Recipients for embryo transfer will be rendered pseudo pregnant by mating with a sterile (vasectomized) male.
   b) Genetically modified embryos will be implanted surgically or non-surgically into the reproductive tract.
c) Embryo recipients, not as part of an experiment, will be killed after weaning of the pups at three weeks of age.

3) Weaned pups at 3 weeks of age: Tissue sampling for genotyping and/or identification via tail and earcut, respectively, under anesthesia (isoflurane).

Animals are killed by O2/CO2 method.

Welfare assessment:
We will daily check the mice on several parameters (overall appearance, size, confirmation and growth, coat condition, behavior, clinical signs, relative size and numbers) as has been described in the Directive 2010/63/EU: corrigendum of 24 Jan. 2013.

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

Statistical analysis doesn't play a role for these types of experiments. We will use state of the art techniques. All techniques are proven to be effective in generating GM mice with a minimum number of mice possible.

B. The animals

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

Mus musculus: genetically modified and wild type adult mice. All vasectomized males which will be obtained from a registered commercial company, all other mice are derived from our own Institute, an establishment licensed by the NVWA, or from a registered commercial company.

Generation of GG mice: we expect, based on our experience of the last 10 years, to generate max. 70 new lines over the nest 5 years. For the creation of a new GM mouse line we will use on average max. 150 mice (according to the besluit biotechnologie). Therefore in total max. 10.500 mice.

Welfare assesment: we expect to generate over the next 5 years 80 new (compound) GM lines for which we have to perform the welfare assesment. For 2 generations, 7 males and 7 females control and GM mice. We therfore need in total: 80 (new (compound) lines) * 2 (generation) * 28 ((7 male +7 female = 14 GM mice + (7 male + 7 female = 14 control mice))= 4480 mice.

Therefore in total max. 10.500 (generation of GGM) + 4480 (welfare assesment) = 14.980 mice
Of note
The majority of the newly generated GG mice will be floxed mice which are not part of the welfare assesment protocol.

We will not breed/kill mice showing a hampered phenotype.

C. Re-use

Will the animals be re-used?

☐ No, continue with question D.

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

- (Vasectomized) males will be 'shared' with the other groups of the Hubrecht Institute, thereby reducing the number of (vasectomized) males used for the generation of GM mice.
  Mice used for welfare assessment, might be used for experiments described in procedures 3.4.4.2-3.4.4.5.

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.

Before we consider the generation of a new (compound) GM Mice we first will extensively analyze cell lines, existing tissue patient material and/or organoids. Only if the identified genes showed an interesting phenotype in these in vitro experiments, we will consider the generation of a novel GM mice.
Animal studies are unavoidable if we seek comprehensive knowledge and understanding of gene function during development and in adult tissue homeostasis.
The CRISPR/Cas9 system allows us, if required, to genetically modify up to 5 different genes in a single experiments. This strongly reduce the number of mice used for the generation and/or breeding of these compound mice.

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

All possibilities to reduce pain, fear or suffering will be used. There are no negative environmental effects; all mice will be housed under strict D1 conditions

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

The proposed procedures are just fundamental research, it does not consist of legally required research.

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?

☒ 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?

☒ 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 1.

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

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

☒ Yes

I. Other aspects compromising the welfare of the animals

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

We don´t expect to find other adverse effect. This is the direct result of how we create our contsructs for the generation of GM mice

Explain why these effects may emerge.

We don´t expect to find other adverse effect.

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

We daily monitor our mice; action will be taken immediately if unexpectedly any adverse effect will show up.

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.

☐ 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').

Donors: mild 100%
Fosters: moderate 100%
GM mice: no to mild 100%

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No > Continue with Section 3: 'signatures'.

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

The donor females will be killed as part of the experiments.
The foster females will be killed after the experiment (at weaning of the pups)

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

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

☒ Yes

Appendix
Description animal procedures

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- 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'.	80102
1.2	Provide the name of the licenced establishment.	KNAW-Hubrecht Institute - Developmental Biology and stem cells
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 2 Type of animal procedure: Tissue, lineage and/or cell type specific gene (in)activation and (over)(mis)expression.

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.

Our aim is to determine the role of stem cells and their derivatives during development and in adult tissue homeostasis via the analysis of (compound) mouse models.

We will use inducible systems including e.g. the well-known Cre-LoxP system in which a floxed gene can be deleted, overexpressed or misexpressed upon the induction of the Cre enzyme via the administration of tamoxifen. In particular, when Cre is expressed in mice harboring a LoxP-containing target gene, the desired gene modification can be restricted to certain developmental stage, organ (e.g. intestine), cell type (e.g. stem cell) of the mouse depending on the specificity and timing of recombinase expression. Successful deletion/activation via (e.g.) Cre enzyme induction might be monitored via expression of a reporter gene (e.g. LacZ or fluorescent protein(s)).
The presence of e.g. a fluorescent marker in a (putative) stem cell allows us to localize these cells by histology and to isolate the fluorescent expressing cells via FACS sorting, which allows us to analyze (gene expression profile) and culture these cells.
The introduction of e.g. a toxin receptor (e.g., Diphtheria toxin receptor) in the cells allows us to specifically kill these cells upon the administration of the toxin (e.g., Diphtheria toxin). This study allows us to determine the consequence of the loss of the toxin receptor expressing cells during development and for tissue homeostasis.
The administration of small molecule compounds/drugs/chemicals/toxins, we might be able to rescue or mimic the phenotypes of the in vivo genetic deletion(s) and/or activation(s) and therefore further identify the function of these cells in vivo.
The mice might be injected with DNA labelling agents prior to killing to measure the proliferation capacity of the stems and their derivatives.
In all experiments, animals will be killed and embryos, neonatal and/or adult organs will be recovered for detailed analysis of the consequences of the genetic alteration and/or treatment on the (developing) tissue(s). Analysis will include among others histological sections labelled with antibodies or antisense RNA probes, RNA expression analysis, DNA or protein extracts. Also, cells from organs might be isolated by FACS and/or cultured in vitro (organoids).

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

1) Tissue sampling for genotyping and identification via ear and tail biopsy resp. under anesthetics (isoflurane).
2) Administration of transgene inducing or deleting agents or control substances alone or in combination, continuously or intermittently by one or more of the following routes:
   a) in diet or drinking water (max. 1 time, max. 2wks)
   b) subcutaneous (max. 1 time)
   c) intraperitoneal (max. 5 times)
   d) implantation of a slow release pellet or pump subcutaneously under adequate anesthesia and analgesia
   e) oral gavage (max. 1 time)

3) (Optional) Administration of small molecule compounds, drugs, toxin, chemicals or control substances alone or in combination, continuously or intermittently by one or more of the following routes:
   a) in diet or drinking water (1 time, <2wks)
   b) subcutaneous (1 time)
   c) intraperitoneal (max. 5 times)
   d) implantation of a slow release pump subcutaneously under adequate anesthesia and analgesia
   e) oral gavage (max. 10 times)

4) (Optional) Administration of a labelling agent (e.g. BrdU) via one of the following routes:
   a) intraperitoneal (max. 1 time)
   b) implantation of a slow release pellet or pump subcutaneously under adequate anesthesia and analgesia
   c) intravenous (max. 1 time)

5) Animals are killed by:
a) Adult mice: via CO2/O2 method or perfusion fixation under lethal dose of Nembutal.
b) Embryo's and neonates: will be put on icewater (but not in direct contact) for 10 min. after which they will be decapitated and the head immediately frozen (or the brains dissected and fixed).

6) Organ(s)/Tissue will be isolated

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

Quantitative analysis: prior to performing an experiment we perform statistical analysis (power analysis) to ensure that we use the minimum number of mice per group that will be statistically sound and biological relevant.
Qualitative analysis (most of our experiments): the number is based in literature and/or years of experience with similar type of experiments Moreover, these types of experiments will be performed sequentially via which we ensure that we will use the minimum number of mice per group that will be informative.
Only mice with the desired genotype will be used.

B. The animals

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

Mus musculus, genetically modified
Origin: GG mice: our own breedings at the Hubrecht institute.
Embryos: max. 2.000
Neonates: max. 1.000
Adult: max. 4.000

The number of mice per experiment will ultimately be based on statistical analysis (power analysis), our experience with similar type of experiments and/or (un)published data.
It is currently absolutely impossible to exactly mention the number of mice required for these experiments over the next 5 years or per experiment. This strongly depends on the type of experiment we want to perform in combination with the specific characteristics of certain (stem)cells within a specific organ. E.g., the number of mice required for expression profiling is strongly of depending on the number of stem cells present in that organ. Indeed, for complete expression profiling of intestinal stem cell we nowadays only need 10 mice, while for a rare stomach stem cell we need 115 mice.
For these reasons we have calculated the total number of mice based on experience over the past 5 years with similar type of experiments.
Importantly, before we will start our experiments we will write an application to the IVD. In this application we will exactly describe (among others) which considerations, facts and results have led to the proposition of the experiments, which specific question(s) we are trying to answer with the proposed animal experiments and what the ultimate goal is for the proposed experiments. Moreover we will describe the experimental design, argue the number of animals in the experiments, describe (i.a.) human endpoints, alternatives, nature of discomfort and ethical considerations. Experiments will only start upon IVD approval.

C. Re-use

Will the animals be re-used?

☒ 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.

Before we consider the import or generation and subsequently analysis of (compound) GGM we first will extensively analyze cell lines, existing tissue patient material and/or organoids. However, animal studies are unavoidable if we seek comprehensive knowledge and understanding of gene function during development and in adult tissue homeostasis.
The necessary animal studies in this project will exclusively involve mice. In that regard, the knowledge and expertise accumulated from the investigation of the mouse is incomparable. Up today, there are no alternative methods to fully understand developmental and adult tissue homeostasis in the context of the whole organism. For the majority of the proposed studies, the mouse is the most appropriate animal model because: (1) it is a mammal; (2) physiology is more extensively characterized in mice than in other mammalian model species; (3) mice are amenable to transgenic manipulation; (4) a large number of relevant transgenic and knock out lines are already available.
Nevertheless, our research will make extensive use of the organoid culture technology we have developed, which by itself extensively reduces the animal numbers for these experiments. The use of this near-native culture system allows this project to comply with the 3Rs (replacement, reduction and refinement) by keeping the mice numbers to be used to minimum.
Whenever possible, we will perform pilot studies with the minimum amount of animals possible.
Mice with inducible alleles will be used, so mice should not display a phenotype before the induction of the alleles.
Experiments will be done sequential.
If homozygous mice doesn't show any discomfort we will keep the mice on a homozygous background, thereby reducing the number of mice.
We will only use well-established reagents and protocols to induce expression or deletion of the candidate gene/s.

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

All possibilities will be used reduce pain, fear or suffering. There are no negative environmental effects; all mice will be housed under strict D1 regulations.

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

The proposed procedures are just fundamental research, it does not consist of legally required research.

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?

☒ 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?

☒ 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 1.

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

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

☒ Yes

I. Other aspects compromising the welfare of the animals

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

Other than in terminally anaesthetized animals dosing and sampling procedures will be undertaken using a combination of volumes, routes and frequencies that will result in no more than transient, mild discomfort.
Due to administration of inducing agents or other substances animals will be experiencing no follow up effects.

It is expected that no animals (0%) will be experiencing more then mild discomfort due to the genetic modification. However, it is impossible to predict the nature or severity of any potential genetic defect. So, in all cases, animals will be careful monitored for possible side effects. Animals exhibiting any unexpected harmful phenotypes will be killed within a day.

Explain why these effects may emerge.

The impairment of normal epithelial function due to genetic alterations and/or administration of small molecule compounds/drugs/chemicals/toxins.

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

Daily monitoring of the mice combined with experimental design.

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.

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

Weight loss (>20% in comparison with control), changes in behavior or body posture, signs of general sickness and/or discomfort.

Indicate the likely incidence.

expected 0% within time frame of the experiments.

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').

Expected:
Embryo's: mild 100%
Neonates: mild 100%
Adult: mild 99%, 1% moderate <1day (placing slow release pump)

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No > Continue with Section 3: 'signatures'.

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

The mice will be killed as part of the experiments.

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

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

☒ Yes

Appendix
Description animal procedures

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1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	80102
1.2	Provide the name of the licenced establishment.	KNAW-Hubrecht Institute - Developmental Biology and stem cells
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 3 Type of animal procedure: Lineage tracing and regeneration

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.

Our aim is to perform lineage tracing studies of stem cells and their derivatives in normal tissue homeostasis, in genetically altered mice and during regeneration upon induced injury.

Lineage tracing provides information about the number of progeny of the founder cell, their location and their differentiation status. Genetic lineage tracing in mice is nowadays usually performed using the well-known Cre-loxP system. Creert2 recombinase is expressed under the control of e.g. (stem)cell-specific promoter in one mouse line crossed with a reporter line.
Tracing might be performed in all tissue such as liver, pancreas, ovary, lung, prostate, kidney, brain, etc of genetically modified mice.
The introduction of e.g. a toxin receptor in (a) cell(s) (type) allows us to specifically kill these cells upon the administration of the toxin. This type of experiments allows us to perform lineage tracing studies in the absence of the toxin receptor expressing cell.
Successful deletion/activation via (e.g.) Cre enzyme induction might be monitored via expression of a reporter gene (e.g. LacZ or fluorescent protein(s)).
I.a. injury will be inflicted via the administration of small molecule compounds/drugs/chemicals/toxins or irradiation, partial ectomy or ischemie and perfusion.
The presence of e.g. a fluorescent marker in cells allows the isolation of the fluorescent expressing cells via FACS sorting, which allows us to analyze (gene expression profile) and culture these cells.
The regenerative response might be stimulated via the administration of validated chemicals, drugs and/or proteins.
The mice might be injected with DNA labelling agents prior to killing to measure the proliferation capacity of the stems and their derivatives.

In all experiments, embryos, neonates and adult animals will be killed and organs will be recovered for detailed analysis of the consequences of the regeneration process in normal and/or inflicted tissue damage of GM mice. Analysis will include among others histological sections labelled with antibodies or antisense RNA probes, RNA expression analysis, DNA or protein extracts. Also, cells from organs might be isolated by FACS and cultured in vitro (organoids). Occasionally, we will perform imaging studies and/or withdraw blood samples.

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

This procedure may consist of the following steps:
1) Tissue sampling for genotyping and identification via ear and tail biopsy resp. under anesthetics (isoflurane).

2) Administration of transgene inducing or deleting agents or control substances alone or in combination, continuously or intermittently by one or more of the following routes:

a) in diet or drinking water (max. time, <2 weeks)
b) subcutaneous (max. 1 time)
c) intraperitoneal (max. 5 times)
d) implantation of a slow release pump subcutaneously under adequate anesthesia and analgesia (max. 1time)
e) oral (max. 1 times)

3) (Optional) Inflicting tissue damage via administration of small molecule compounds, drugs, toxin, chemicals or control substances alone or in combination, continuously or intermittently by one or more of the following routes:
a) in diet or drinking water (max. 1 time, <2 weeks)
b) subcutaneous (max. 1 time)
c) intraperitoneal (max. 5 times)
d) implantation of a slow release pump subcutaneously under adequate anesthesia and analgesia
e) oral (max. 10 times)
f) topical application (max. 3 times)

OR via
g) irradiation (none lethal dose, max. 1 time)
i) ectomy of a part of a specific organ (max. 1 time) under adequate anesthesia and analgesia.
j) Ischemie and reperfusion (max. 1 time) under adequate anesthesia and analgesia.

4) (Optional) Administration of validated chemical, drugs, control substances and/or proteins for stimulation of the regenerative response alone or in combination, continously or intermittently by one or more of the following routes:
a) in diet or drinking water (max. time, <2 weeks)
b) subcutaneous (max. 1 time)
c) intraperitoneal (max. 5 times)
d) implantation of a slow release pump subcutaneously under adequate anesthesia and analgesia (max. 1time)
e) oral (max. 1 times)

5) (Optional) Withdrawal of blood sample(s) (max. 10 times) without anesthesia.

6) (Optional) Implantation of a window under proper anesthesia and analgesia. Imaging by a light source (e.g. fluorescence or luminescence) typically for not longer than 20 min. per imaging sessions under isoflurane anesthesia for not more than twice a week during a maximum period of 90. for organs of which imaging methods are already developed at the Hubrecht Institute. Administration of substances (e.g. luciferin) may be necessary before or during imaging by one of the following routes: subcutaneous or intraperitoneal.

7) (Optional) Administration of a labelling agent (e.g. BrdU) via one of the following routes:
a) intraperitoneal (max. 1 time)
b) implantation of a slow release pump subcutaneously under adequate anesthesia and analgesia
c) intravenous (max. 1 time)

8) Animals may be killed by:
a) Adult mice: via CO2/O2 method or perfusion fixation under lethal dose of Nembutal.
b) Embryo's and neonates: will be put on melting icewater for 10 min. (but not in contact with) after which they will be decapitated and the head immediately frozen (or the brains dissected and fixed).

9) Isolation of organs from killed mice.

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

Quantitative analysis: prior to performing an experiment we perform statistical analysis (power analysis) to ensure that we use the minimum number of mice per group that will be statistically sound and biological relevant.
Qualitative analysis (most of our experiments): the number is based in literature and/or years of experience with similar type of experiments Moreover, these types of experiments will be performed sequentially via which we ensure that we will use the minimum number of mice per group that will be informative.
Only mice with the desired genotype will be used.

B. The animals

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

Mus musculus: genetically modified; the mice are obtained from our own breedings or from a commercial licensed breeder.
Embryo's: max. 2.000
Neonates: max. 1.000
Adult: max. 4.000

The number of mice per experiment will ultimately be based on statistical analysis (power analysis), our experience with similar type of experiments and/or (un)published data.
It is currently absolutely impossible to exactly mention the number of mice required for these experiments over the next 5 years or per experiment.
The number of mice required for lineage tracings studies strongly depends on the organ and/or number of targeted cells and/or expression level of the Cre enzyme etc. For lineage tracing studies of adult intestinal lgr5+ stem cells we should nowadays need 98 mice (6 mice per group (5+1 control), 8 time points (to follow the tracing over time) * 2 (2 independent methods for fixation of the tissue which allows us to perform a complete and thorough analysis).
However, the number of mice required for the proper analysis of e.g. stem cells in other organs can be different due to their specific characteristics and/or dynamics.
For these reasons we have calculated the total number of mice based on experience over the past 5 years with similar type of experiments.
Importantly, before we will start our experiments we will write an application to the IVD. In this application we will exactly describe (among others) which considerations, facts and results have led to the proposition of the experiments, which specific question(s) we are trying to answer with the proposed animal experiments and what the ultimate goal is for the proposed experiments. Moreover we will describe the experimental design, argue the number of animals in the experiments, describe (i.a.) human endpoints, alternatives, nature of discomfort and ethical considerations. Experiments will only start upon IVD approval.

C. Re-use

Will the animals be re-used?

☒ 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.

Before we consider the import or generation and subsequently analysis of (compound) GGM we first will extensively analyze cell lines, existing tissue patient material and/or organoids. However, animal studies are unavoidable if we seek comprehensive knowledge and understanding of gene function during development and in adult tissue homeostasis.
The necessary animal studies will exclusively involve mice. In that regard, the knowledge and expertise accumulated from the investigation of the mouse is

incomparable. Up today, there are no alternative methods to fully understand developmental and adult tissue homeostasis in the context of the whole organism. For the majority of the proposed studies, the mouse is the most appropriate animal model because: (1) it is a mammal; (2) physiology is more extensively characterized in mice than in other mammalian model species; (3) mice are amenable to transgenic manipulation; (4) a large number of relevant transgenic and knock out lines are already available.
Nevertheless, this program will make extensive use of the organoid culture technology we have developed, which by itself extensively reduces the animal numbers. The use of this near-native culture system allows to comply with the 3Rs (replacement, reduction and refinement) by keeping the mice numbers to be used to minimum.
Whenever possible, we will perform pilot studies with the minimum amount of animals possible.
Mice with inducible alleles will be used, so mice should not display a phenotype before the induction of the alleles.

Experiments will be done sequential.
If homozygous mice doesn't show any discomfort we will keep the mice on a homozygous background, thereby reducing the number of mice.
We will only use well-established reagents and protocols to induce expression or deletion of the candidate gene/s and/or to inflict the tissue damage.

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

All possibilities will be used reduce pain, fear or suffering. There are no negative environmental effects; all mice will be housed under strict D1 regulations.

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

The proposed procedures are just fundamental research, it does not consist of legally required research.

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?

☒ 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?

☒ 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 1.

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

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

☒ Yes

I. Other aspects compromising the welfare of the animals

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

Other than in terminally anaesthetized animals dosing and sampling procedures will be undertaken using a combination of volumes, routes and frequencies that will result in no more than transient minor discomfort.
Due to administration of inducing agents or other substances animals will be experiencing no follow-up effects .
It is predicted that no animals (0%) will be experiencing a severe adverse effect from the genetic modifications with or without the administartion of the inflicted injury
In all cases, animals will be careful monitored for possible side effects. Animals exhibiting any unexpected harmful phenotypes will be killed within a day.

Explain why these effects may emerge.

The impairment of normal epithelial function and/or inflicted tissue damage due to genetic alterations and/or administration of small molecule compounds/drugs/chemicals/toxins.

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

Daily monitoring of the mice combined with experimental design.

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.

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

Weight loss (>20% in comparison with control), changes in behavior or body posture, signs of general sickness and/or discomfort.

Indicate the likely incidence.

expected 0% within time frame of the experiments.

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').

Embryo's: mild 100%
Neonates: mild 100%
Adult: mild 75%, moderate 20% <1day, moderate 5% max. 3 days

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No > Continue with Section 3: 'signatures'.

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

The mice will be killed as part of the experiments.

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

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

☒ 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).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	80102
1.2	Provide the name of the licenced establishment.	KNAW-Hubrecht Institute- Developmental Biology and stem cells
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 4 Type of animal procedure: Generation and analysis of disease models caused by deregulated stem or niche cells

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 activation of aberrant molecular signaling pathways in adult stem cells and/or their niche resulting in a dysfunctional behavior have been associated with diseases. E.g. the accumulation of gene alterations occurring specifically in adult epithelial stem cells could trigger their malignant transformation which contributes to their sustained growth, survival expansion and metastasis. Insight into stem cells and their niches in term of their function in

pathophysiological condition is therefore of fundamental importance to basic biologist and, ultimately, to treat diseases.
Our aim is to therefore to identify the role of stem cells, their derivatives and their niches in the early stages of pathogenesis (e.g. cancer or diabetes) and how to manipulate these cells c.q. interactions.

The following approaches may be undertaken.
We will set up disease models (e.g. cancer) with existing or novel (procedure. 1) GM mice lines.
We will use inducible systems including e.g. the well-known Cre-LoxP system in which a floxed gene can be deleted, overexpressed or misexpressed upon the induction of the Cre enzyme via the administration of tamoxifen. In particular, when Cre is expressed in mice harboring a LoxP-containing target gene, the desired gene modification can be restricted to certain developmental stage, organ (e.g. intestine), cell type (e.g. stem cell) of the mouse depending on the specificity and timing of recombinase expression. Successful deletion/activation via (e.g.) Cre enzyme induction might be monitored via expression of a reporter gene (e.g. LacZ or fluorescent protein(s)).
The presence of e.g. a fluorescent marker in a (putative) stem cell allows us to localize the cells via histological analysis and isolate the fluorescent expressing cells via FACS sorting, which allows us to analyze (gene expression profile) and culture of these pathogenic cells.
The (combined) introduction of e.g. a toxin receptor in the same cells allows us to specifically kill these cells upon the administration of the toxin. This study allows us to determine the consequence of the loss of the toxin receptor cells on the pathogenic tissue
The administration of small molecule compounds/drugs allows us to change, prevent or inhibit the pathogenic process and therefore further identify the understand pathogenesis in vivo. If possible and/or relevant we will always test these small molecule compounds/drugs/chemicals/toxins on in vitro growing organoids before we might use them for in vivo studies.

In all experiments, animals will be killed and adult organs will be recovered for detailed analysis of the consequences of the genetic alteration and/or treatment on the (developing) tissue(s). Analysis will include among others histological sections labelled with antibodies or antisense RNA probes, RNA expression analysis, DNA or protein extracts. Also, cells from organs might be isolated by FACS and/or cultured in vitro (organoids).

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

1) Tissue sampling for genotyping and identification via ear and tail biopsy resp. under anesthetics (isoflurane).
2) Administration of transgene inducing or deleting agents or control substances alone or in combination, continuously or intermittently by one or more of the following routes:
   a) in diet or drinking water (max. 1 time, < 2 wks)
   b) subcutaneous (max. 1 time)
   c) intraperitoneal(max. 5 times)
   d) implantation of a slow release pump subcutaneously under adequate anesthesia and analgesia
   e) oral (max. 10 times)

3) (Optional) Administration of small molecule compounds, drugs, toxin, chemicals or control substances alone or in combination, continuously or intermittently by one or more of the following routes:
   a) in diet or drinking water (max. time, <2 wks)
   b) subcutaneous (max. time)
   c) intraperitoneal (max. 5 times)
   d) implantation of a slow release pellet subcutaneously under adequate anesthesia and analgesia (1 time)
   e) oral (max. 10 times)

4) (Optional) Administration of a labelling agent (e.g. BrdU) via one of the following routes:
a) intraperitoneal (1 time)
b) implantation of a slow release pump subcutaneously under adequate anesthesia and analgesia (1 time)
c) intravenous (1 time)

5) Animals are killed by:
a) Adult mice: via CO2/O2 method or perfusion fixation under lethal dose of Nembutal.

6) Organ(s)/tissue will be isolated

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

Quantitative analysis: prior to performing an experiment we perform statistical analysis to ensure that we use the minimum number of mice per group that will be statistically sound and biological relevant.
Qualitative analysis (most of our experiments): the number is based in literature and/or years of experience with similar type of exp. Moreover, these types of experiments will be performed sequentially via which we ensure that we will use the minimum number of mice per group that will be informative

B. The animals

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

Mus musculus: genetically modified; The mice are obtained from our own breedings.
Adult: max. 4.000
The number of mice per experiment will be based on statistical analysis (power analysis), our experience with similar type of experiments and/or (un)published data.

The number of mice per experiment will ultimately be based on statistical analysis (power analysis), our experience with similar type of experiments and/or (un)published data.
It is currently absolutely impossible to exactly mention the number of mice required for these experiments over the next 5 years or per experiment.
The number of mice required for the analysis of a disease model strongly depends on e.g. the specific characteristic of a certain organ, how many genes we want to (in)activate etc. E.g. in the case of generation tumors in the intestine via the inactivation of a single gene (e.g. Apc) in the stem cells (via lgr5-ires-creert) 60 mice are required for a complete and thorough analysis (5 (number of mice per group) * 6 (different time points require to follow the development of intestinal tumors over time) * 2 (2 independent methods for fixation of the tissue which allows us to perform a complete and thorough analysis). However, the (simultaneously) (in)activation of 2 genes requires 180 mice. Moreover, the number of mice required for proper analysis strongly depends on many factors including characteristics and dynamics of e.g. stem cells of the organ.
For these reasons we have calculated the total number of mice based on experience over the past 5 years with similar type of experiments.
Importantly, before we will start our experiments we will write an application to the IVD. In this application we will exactly describe (among others) which considerations, facts and results have led to the proposition of the experiments, which specific question(s) we are trying to answer with the proposed animal experiments and what the ultimate goal is for the proposed experiments. Moreover we will describe the experimental design, argue the number of animals in the experiments, describe (i.a.) human endpoints, alternatives, nature of discomfort and ethical considerations. Experiments will only start upon IVD approval.

C. Re-use

Will the animals be re-used?

☒ 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.

Before we consider the import or generation and subsequently analysis of (compound) GGM to study disease models, we first will extensively analyze cell lines, existing tissue patient material and/or organoids. However, animal studies are unavoidable if we seek comprehensive knowledge and understanding of gene function and their manipulation during pathogenesis.
The necessary animal studies in this project will exclusively involve mice. In that regard, the knowledge and expertise accumulated from the investigation of the mouse is incomparable. Up today, there are no alternative methods to fully understand developmental and adult tissue homeostasis in the context of the whole organism. For the majority of the proposed studies, the mouse is the most appropriate animal model because: (1) it is a mammal; (2) physiology is more extensively characterized in mice than in other mammalian model species; (3) mice are amenable to transgenic manipulation; (4) a large number of relevant transgenic and knock out lines are already available.
Nevertheless, this program will make extensive use of the organoid culture technology we have developed, which by itself extensively reduces the animal numbers. The use of this near-native culture system allows this project to comply with the 3Rs (replacement, reduction and refinement) by keeping the mice numbers to be used to a minimum.
Whenever possible, we will perform pilot studies with the minimum amount of animals possible.
The mice with inducible alleles will be used, so mice should not display a phenotype before the induction of the alleles.
Experiments will be done sequential.
If homozygous mice doesn't show any discomfort we will keep the mice on a homozygous background, thereby reducing the number of mice.
We will only use well-established reagents and protocols to induce expression or deletion of the candidate gene/s.

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

All possibilities will be used to reduce pain, fear or suffering. There are no negative environmental effects; all mice will be housed under strict D1 regulations.

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

The proposed procedures are just fundamental research, it does not consist of legally required research.

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?

☒ 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?

☒ 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 1.

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

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

☒ Yes

I. Other aspects compromising the welfare of the animals

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

Other than in terminally anaesthetized animals dosing and sampling procedures will be undertaken using a combination of volumes, routes and frequencies that will result in no more than transient minor discomfort.
Due to administration of inducing agents or other substances animals will be experiencing no follow-up effects .
It is predicted that no animals (0%) will be experiencing a severe adverse effect from the genetic modifications with or without the administartion of the inflicted injury
In all cases, animals will be careful monitored for possible side effects.
Animals exhibiting any unexpected harmful phenotypes will be killed within a day.

Explain why these effects may emerge.

The impairment of normal epithelial function damage due to genetic alterations and/or administration of small molecule compounds/drugs/chemicals/toxins.

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

Daily monitoring of the mice combined with experimental design.

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.

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

Weight loss (>20% in comparison with control), changes in behavior or body posture, signs of general sickness and/or discomfort.

Indicate the likely incidence.

expected 0% within time frame of the experiment.

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').

Adult, mild 99%, moderate 1% <1 day (placing slow release pump)

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No > Continue with Section 3: 'signatures'.

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

The mice will be killed as part of the experiments.mild 80% <1 day, moderate 20% <1 day

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

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

☐ 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).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	80102
1.2	Provide the name of the licenced establishment.	KNAW-Hubrecht Institute Developmental Biology and Stem cells
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 5 Type of animal procedure: Growth and genetically modification of organoids and (xeno) transplantation.

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.

Our aim is to determine whether we can use the wild type and/or functionally repaired and/or genetically modified organoids as a tool for adult stem cell-based gene therapy. We will therefore perform (xeno)transplantation of wild type and/or functionally repaired and/or genetically modified organoid in diseased, damaged or wild type organs. Experiments will only be carried if validated (disease) models exist.

We will use inducible systems including e.g. the well-known Cre-LoxP system in which a floxed gene can be deleted, overexpressed or misexpressed upon the induction of the Cre enzyme via the administration of tamoxifen.
Successful deletion/activation via (e.g.) Cre enzyme induction might be monitored via expression of a reporter gene (e.g. LacZ or fluorescent protein(s)).
The introduction of e.g. a toxin receptor in (a) cell(s) (type) allows us to specifically kill these cells upon the administration of the toxin. This type of experiments allows us to perform transplantation studies in the absence of the toxin receptor expressing cell.
I.a. injury will be inflicted via the administration of small molecule compounds/drugs/chemicals/toxins, ectomy of a part of a specific organ or irradiation.
The presence of e.g. a fluorescent marker in cells allows the isolation of the fluorescent expressing cells via FACS sorting, which allows us to analyze (gene expression profile) and culture these cells.
The mice might be injected with DNA labelling agents prior to killing to measure the proliferation capacity of the stems and their derivatives.
Occasionally, we will perform imaging studies and/or withdrawal of bloodsamples

In all experiments, adult animals will be killed and organs will be recovered for detailed analysis of the consequences of the regeneration process in normal and/or inflicted tissue damage of GM mice. Analysis will include among others histological sections labelled with antibodies or antisense RNA probes, RNA expression analysis, DNA or protein extracts. Also, cells from organs might be isolated by FACS and cultured in vitro (organoids).

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

This procedure will consist of the following steps:
1) Tissue sampling for genotyping and identification via ear and tail biopsy resp. under anesthetics (isoflurane).

2) Administration of transgene inducing or deleting agents or control substances alone or in combination, continuously or intermittently by one or more of the following routes:
- a) in diet or drinking water (max. 1 time, max. 2 wks)
- b) subcutaneous (max. 1 time)
- c) intraperitoneal (max. 5 times)
- d) implantation of a slow release pellet subcutaneously under adequate anesthesia and analgesia (1 time).
- e) oral (max. 1 time)

3) (Optional) Inflicting tissue damage via administration of small molecule compounds, drugs, toxin, chemicals or control substances alone or in combination, continuously or intermittently by one or more of the following routes:
- a) in diet or drinking water (1 time, max. 2 wks)
- b) subcutaneous (1 time)
- c) intraperitoneal (max. 5 times)
- d) implantation of a slow release pellet subcutaneously under adequate anesthesia and analgesia (1time)
- e) oral (max. 10 times)
- f) topical application (max. 5 times)

OR
- g) irradiation (max. 1 time)
- h) ischemie and reperfusion under adequate anesthesia and analgesia (1 time)

4) Administration of wild type and/or functionally repaired and/or genetically modified organoid in diseased, damaged or wild type organs under adequate anesthesia and analgesia

5) (Optional) Withdrawal of blood samples without anesthesia.

6) (Optional) Implantation of window under adequate anesthesia and analgesia. Imaging by a light source (e.g. fluorescence or luminescence) typically for not longer than 20 min per imaging sessions under isoflurane anesthesia for not more than twice a week during a maximum period of 90 days for which the administration of substances (e.g. luciferin) may be necessary before or during imaging by one of the following routes: subcutaneous or intraperitoneal.

7) (Optional) Administration of a labelling agent (e.g. BrdU) via one of the following routes:
   a) intraperitoneal (max. 1 time)
   b) implantation of a slow release pump subcutaneously under adequate anesthesia and analgesia (max. 1 time)
   c) intravenous (max. 1 time)

8) Animals will killed by:
   a) Adult mice: via CO2/O2 method or perfusion fixation under lethal dose of Nembutal.

9) Isolation of organ(s)/tissue.

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

Quantitative analysis: prior to performing an experiment we perform statistical analysis (power analysis) to ensure that we use the minimum number of mice per group that will be statistically sound and biological relevant.
Qualitative analysis (most of our experiments): the number is based in literature and/or years of experience with similar type of exp. Moreover, these types of experiments will be performed sequentially via which we ensure that we will use the minimum number of mice per group that will be informative.

B. The animals

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

Mus musculus: genetically modified and wild type; The mice are obtained from our own breedings or from a commercial licensed breeder.
Adult: max. 1.600
The number of mice per experiment will ultimately be based on statistical analysis (power analysis), our experience with similar type of experiments and/or (un)published data.
It is currently absolutely impossible to exactly mention the number of mice required for these experiments over the next 5 years or per experiment. For many organs we first have to optimize/find the correct transplantations conditions. Eg. we only needed 30 mice for setting up our liver transplantation protocols, while for the small intestines we still trying to find the correct condition for transplantation. Moreover, the type and number of experiments are strongly depending on the number of wild type, genetically manipulated and/or gene corrected and/or diseased organoids we want to transplant per organ.
For these reasons we have calculated the total number of mice based on experience over the past 5 years with similar type of experiments.
Importantly, before we start our experiments we will write an application to the IVD. In this application we will exactly describe (among others) which considerations, facts and results have led to the proposition of the experiments, which specific question(s) we are trying to answer with the proposed animal experiments and what the ultimate goal is for the proposed experiments. Moreover we will describe the experimental design, argue the number of animals in the experiments, describe (i.a.) human endpoints, alternatives, nature of discomfort and ethical considerations. Experiments will only be started upon IVD

approval.

C. Re-use

Will the animals be re-used?

☒ 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.

The necessary animal studies in this project will exclusively involve mice. In that regard, the knowledge and expertise accumulated from the investigation of the mouse is incomparable. Up today, there are no alternative methods to fully understand developmental and adult tissue homeostasis in the context of the whole organism. For the majority of the proposed studies, the mouse is the most appropriate animal model because: (i) it is a mammal; (ii) physiology is more extensively characterized in mice than in other mammalian model species; (iii) mice are amenable to transgenic manipulation; (iv) a large number of relevant transgenic and knock out lines are already available.
Whenever possible, we will perform pilot studies with the minimum amount of animals possible.
I.a. mice with inducible alleles will be used, so mice should not display a phenotype before the induction of the alleles.
Experiments will be done sequential.
If homozygous mice doesn't show any discomfort we will keep the mice on a homozygous background, thereby reducing the number of mice.
We will only use well-established reagents and protocols to induce expression or deletion of the candidate gene/s and/or inflict injury.

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

All possibilities will be used to reduce pain, fear or suffering. There are no negative environmental effects; all mice will be housed under strict D1 regulations.
If possible transplantation conditions will first be validated in wild type animals before we will perform organoid transplantation in GG mice with injured and/or diseased organs.

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

The proposed procedures are just fundamental research, it does not consist of legally required research.

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?

☒ 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?

☒ 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 1.

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

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

☒ Yes

I. Other aspects compromising the welfare of the animals

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

Other than in terminally anaesthetized animals dosing and sampling procedures will be undertaken using a combination of volumes, routes and frequencies that will result in no more than transient discomfort and no lasting harm.
Due to the administration of inducing agents or other substances animals will be experiencing no follow up effect.
It is therefore expected that no animals (0%) will be experiencing a severe adverse effect.
In all cases, animals will be careful monitored for possible side effects.
Animals exhibiting any unexpected harmful phenotypes will be killed within a day.

Explain why these effects may emerge.

The impairment of normal epithelial function due to genetic alterations and/or administration of small molecule compounds/drugs/chemicals/toxins .

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

Daily monitoring of the mice combined with experimental set up (see I).

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.

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

Weight loss (>20% in comparison with control), changes in behavior or body posture, signs of general sickness and/or discomfort.

Indicate the likely incidence.

expecetd 0% within time frame of the experiment

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').

moderate 85% <1day, 10% <3 days, 5% <90days.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No > Continue with Section 3: 'signatures'.

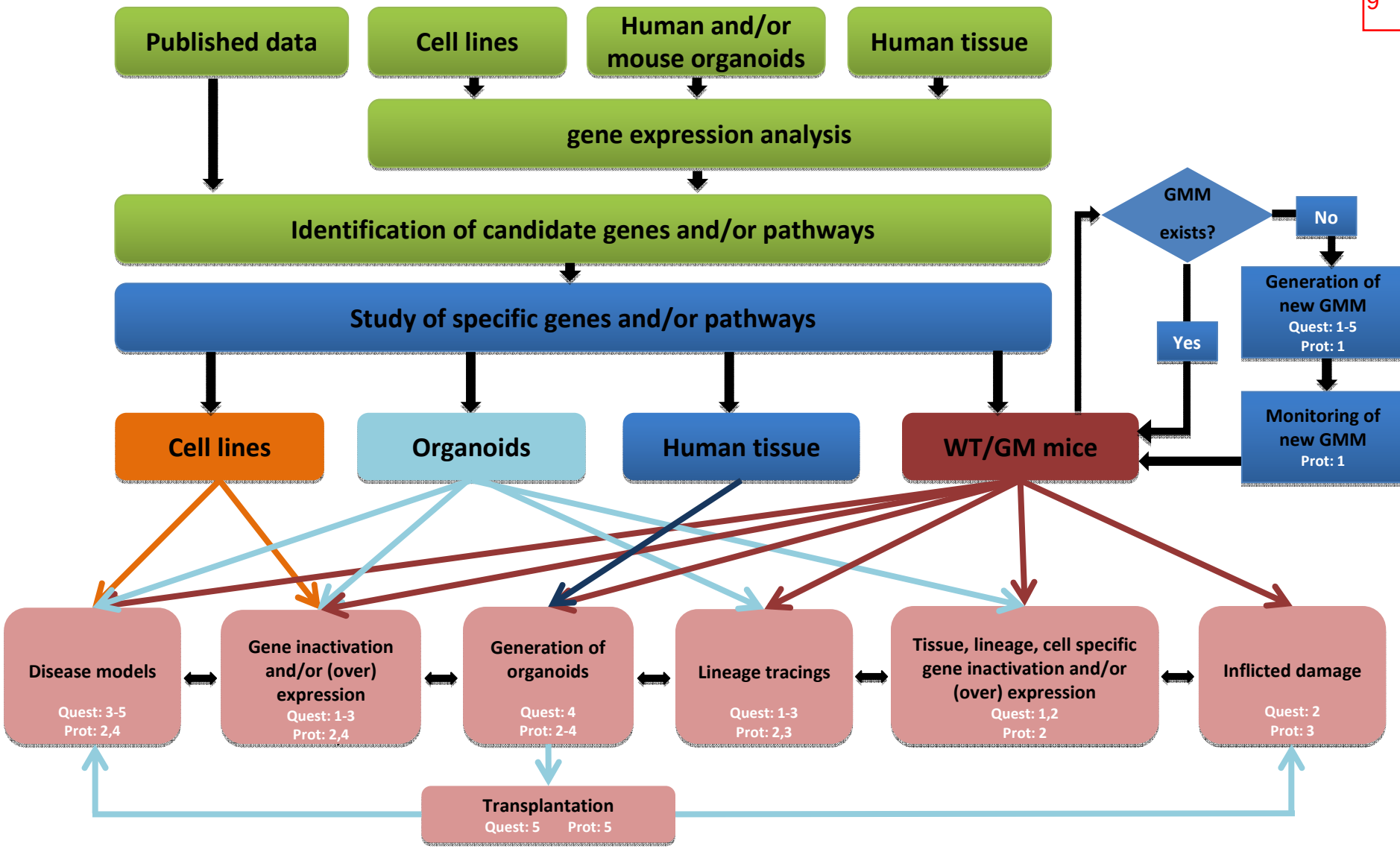
☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

The mice will be killed as part of the experiments.

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

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

☒ Yes



Output:

1) New insights into the role of stem cells and their derivatives during development and in tissue homeostasis.
2) New insights into the role of stem cells and their derivatives in regeneration.
3) New insights into the role of stem cells, their derivatives and their niches during pathogenesis and the development of methods to manipulate, prevent or alleviate these (pathogenic) cells.
4) Optimization of growth conditions for culturing organoids derived from organs of genetically modified (compound) and wild type mice.
5) The development and optimization of methods for usage of wild type, functionally repaired or genetically modified organoids as a tool in (regenerative) medicine.

11 januari 2015

Format DEC-advies

Maak bij de toepassing van dit format gebruik van de bijbehorende toelichting, waarin elke stap in het beoordelingsproces wordt toegelicht

A. Algemene gegevens over de procedure

1. Aanvraagnummer: AVD8010020151
2. Titel van het project: The role of stem cells and their derivatives during development, adult tissue homeostasis, diseases and regenerative medicine.
3. Titel van de NTS: Adulte stamcellen: van identificatie tot regeneratieve geneeskunde.
4. Type aanvraag:
   - ✓ nieuwe aanvraag projectvergunning
   - ☐ wijziging van vergunning met nummer
5. Contactgegevens DEC:
   - naam DEC: KNAW
   - telefoonnummer contactpersoon: [redacted]
   - mailadres contactpersoon: [redacted]
6. Adviestraject (data dd-mm-jjjj):
   - ✓ ontvangen door DEC: 18-01-2015
   - ✓ aanvraag compleet: 06-02-2015
   - ✓ in vergadering besproken: 22-01-2015
   - ✓ anderszins behandeld: n.v.t.
   - ✓ termijnonderbreking(en): n.v.t.
   - ☐ besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen:
   - ☐ aanpassing aanvraag:
   - ✓ advies aan CCD: 11 februari 2015
7. Eventueel horen van aanvrager
   - Datum: donderdag 22-01-2015
   - Plaats: Utrecht
   - Aantal aanwezige DEC-leden: 6 leden
   - Aanwezige (namens) aanvrager: Verantwoordelijk Onderzoeker

8. Correspondentie met de aanvrager:
   - Datum 26-01-2015
   - Strekking: completering van de aanvraag
   - Datum antwoord 06-02-2015
   - Strekking van de antwoorden: de aanvraag is gecompleteerd
9. Eventuele adviezen door experts (niet lid van de DEC): geen

B. Beoordeling (adviesvraag en behandeling)

1. Het project is vergunningplichtig. Het omvat dierproeven in de zin der wet.
2. De aanvraag betreft een nieuwe aanvraag. Er is enige overlap met een aantal al van een positief advies voorziene DEC-protocollen.
3. De DEC is competent om over deze projectvergunningsaanvraag te adviseren. De benodigde expertise op dit wetenschappelijk terrein is aanwezig binnen de DEC. Geen van de DEC-leden is betrokken bij het betreffende project.
4. Vanwege betrokkenheid bij het betreffende project is een aantal DEC-leden, met het oog op onafhankelijkheid en onpartijdigheid, niet betrokken bij de advisering: n.v.t.

C. Beoordeling (inhoud):

1. Het project is:

   - ✓ uit wetenschappelijk oogpunt verantwoord
   - □ uit onderwijskundig oogpunt verantwoord
   - □ uit het oogpunt van productiedoeleinden verantwoord
   - □ wettelijk vereist

2. De in de aanvraag aangekruiste doelcategorie is in overeenstemming met de hoofddoelstelling.
3. De doelstelling, in relatie tot de uitvoering, is helder omschreven. De DEC onderschrijft het belang van de doelstelling, te weten; het verkrijgen van fundamenteel wetenschappelijk inzichten in de rol van stamcellen in verschillende organen. Meer specifiek, het verkrijgen van inzichten in de identificatie, isolatie en karakterisering van stamcellen en de identificatie en

karakterisering van de processen die het gedrag van stamcellen en hun afstammelingen en de rol van hun niche onder normale en pathologische condities controleren. Daarnaast richt het project zich op de ontwikkeling van procedures voor het kweken van mini-orgaankweken (organoïden) voor transplantatiedoeleinden en als alternatief voor dierproeven. Het fundamenteel wetenschappelijke belang wordt door de DEC geschat als substantieel. Fundamentele wetenschappelijke kennis van stamcellen is essentieel voor het ontwikkelen van nieuwe en verbeterde therapieën op basis van weefselregeneratie met behulp van stamcellen en dient daarmee een belangrijk maatschappelijk doel.

4. De gekozen strategie, experimentele aanpak in combinatie met de infrastructuur op het Hubrecht Instituut en de expertise van de betrokken onderzoeksgroep bieden een realistisch uitzicht op het behalen van de beoogde doelstellingen binnen gevraagde looptijd van het project. Het project bouwt verder op een langlopende lijn van onderzoek van een grote groep onderzoekers. Over de afgelopen jaren zijn volgens een vergelijkbare strategie en aanpak belangrijke wetenschappelijk resultaten behaald resulterend in vele publicaties in vooraanstaande tijdschriften. Het onderzoek wordt financieel gesteund door veel verschillende onafhankelijke subsidiegevers.
5. Alle dieren worden gefokt voor het gebruik in dierproeven, er is geen sprake van afwijkende huisvesting en/of hergebruik. Er is geen sprake van bedreigde diersoorten, niet-menselijke primaten, zwerfdieren en/of dieren in/uit het wild. De toegepaste methoden voor anesthesie/euthanasie zijn conform de Richtlijn.
6. Het cumulatieve ongerief gepaard gaande met de dierproeven, zoals beschreven in de vijf verschillende type dierproeven, is naar inschatting van de DEC voor het overgrote deel licht ongerief. In sommige omstandigheden zal er sprake zijn van kortdurend matig ongerief (korter dan 1 dag) en in uitzonderlijke gevallen wat langduriger matig ongerief (langer dan 1 dag). Deze inschatting van de DEC is in overeenstemming met het niveau van cumulatief ongerief ingeschat door de onderzoekers. Deze inschatting is gebaseerd op hun ruime ervaring met vergelijkbare dierproeven.
7. Binnen het project wordt maximaal gebruik gemaakt van methoden die de voorgestelde dierproeven geheel of gedeeltelijk **vervangen.** Een belangrijk

onderdeel van de experimentele strategie is de gefaseerde opzet. In de eerste fase, voorafgaand aan de dierproeven, vindt uitgebreid in vitro onderzoek plaats met cellijnen, organoïden en met weefsel afkomstig van eerdere proeven en met patiëntenmateriaal. Na deze fase zijn verschillende go/no-go-beslissingsmomenten voordat tot uitvoering van dierexperimenten wordt besloten.

Binnen dit project zal op grote schaal gebruik gemaakt worden van organoïden. Echter nieuwe inzichten in de ontwikkeling, in de homeostase in volwassen weefsel en de rol van stamcellen in de context van afwijkingen kunnen op dit moment alleen maar verkregen worden in een intact organisme. Naar het oordeel van de DEC zijn er geen alternatieven beschikbaar voor het voorgestelde gebruik van intacte dieren om te doelstelling van dit project te realiseren.

8. In het project wordt optimaal tegemoet gekomen aan de vereisten van **vermindering** van dierproeven. Het merendeel van de experimenten omvat een kwalitatieve analyse van de experimentele resultaten. De onderzoeksgroep heeft jarenlange ervaring met dit soort experimenten en door een veelal gefaseerde opzet wordt per experiment het minimum benodigde aantal dieren ingezet. Voorafgaand aan de kwantitatieve experimenten wordt op basis van literatuurgegevens, eigen historische data of een specifiek hiertoe uitgevoerd pilot experiment de groepsgrootte bepaald. Technieken en procedures worden zorgvuldig toegepast. Dit alles is gebaseerd op jarenlange ervaring. Het maximale aantal te gebruiken dieren is realistisch geschat.
9. De uitvoering van het project is in overeenstemming met de vereisten van **verfijning** van dierproeven en is zo opgezet dat de dierproeven met zo min mogelijk ongerief worden uitgevoerd.
   Bij de opzet wordt rekening gehouden met dierenwelzijn op de volgende manieren: 1) het gebruik van adequate anesthesie en analgesie waar nodig, 2) het gebruik van weefselspecifiek-induceerbare genetisch gemodificeerde muizen, 3) intensieve monitoring van de proefdieren op het optreden van eventueel constitutioneel ongerief van nieuwe gecreëerde genotypes, na inductie van weefselspecifieke genetische modificaties of na de inductie van

schade in de ziektemodellen. Omdat de wetenschappelijke interesse zich bij de ziektemodellen vooral richt op de eerste stadia, bereiken de betrokken dieren eigenlijk nooit stadia met mogelijk meer ongerief.
Er is geen sprake van belangwekkende milieueffecten.

10. De niet-technische samenvatting is een evenwichtige weergave van het project en is geformuleerd in begrijpelijke taal. De NTS voldoet daarmee aan de eisen zoals gesteld in artikel 10.a.1.7 van de Wod.

D. Ethische afweging

Het fundamenteel wetenschappelijke onderzoek in dit project is van substantieel belang en van aangetoonde excellente kwaliteit. De DEC is van mening dat de resultaten zullen bijdragen aan het verkrijgen van een beter wetenschappelijk inzicht in de rol en functie van stamcellen in verschillende organen onder normale condities evenals een beter inzicht in de rol van stamcellen in pathologische omstandigheden. De DEC acht het belang van de fundamenteel wetenschappelijke doelstelling substantieel en schat de kans op het realiseren van de doelstellingen in als hoog. De verkregen fundamenteel wetenschappelijke kennis is essentieel voor verder onderzoek voor een toepassing van stamcellen in regeneratieve therapieën in patiënten met ziektes die veroorzaakt worden door een deregulatie van stamcellen (bijvoorbeeld kanker). Het maatschappelijk belang is daarmee groot.

De onderzoeksgroep beschikt over een ruime ervaring met de gekozen onderzoeksstrategie en met de voorgestelde typen dierproeven.
Bij de het uitvoeren van de dierproeven wordt een adequate invulling gegeven aan de vereisten op het gebied van de vervanging, vermindering en verfijning van de dierproeven. De DEC onderschrijft dat de doelstellingen niet zonder het gebruik van proefdieren kunnen worden behaald en acht het gebruik van het aantal dieren en het daarmee samenhangende grotendeels licht ongerief bij de dieren gerechtvaardigd.

E. Advies

1. Advies aan de CCD

- ✓ **De DEC adviseert de vergunning te verlenen**

2. Het uitgebrachte advies is gebaseerd op consensus.

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Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

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[redacted]
Postbus 19121
1000 GC AMSTERDAM

Centrale Commissie Dierproeven

Postbus 20401
2500 EK Den Haag
www.zbo-ccd.nl

T 0900 28 000 28 (10 ct /min)

info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD8010020151

Bijlagen
Factuur

Datum 25-02-2015
Betreft Factuur Aanvraag projectvergunning dierproeven

Geachte [redacted]

Op 11 februari 2015 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "The role of stem cells and their derivatives during development, adult tissue homeostasis, diseases and regenerative medicine."

Door technische omstandigheden is het momenteel niet mogelijk om uw automatische incasso te innen. Bijgaand treft u daarom de factuur aan voor de betaling van de leges. Wij verzoeken u de leges zo spoedig mogelijk te betalen. Het wijzigen van de betalingswijze, zal de behandeling van uw aanvraag niet vertragen. Is uw betaling niet binnen dertig dagen ontvangen, dan kan een eventuele vergunning worden ingetrokken. Uw eenmalige machtiging komt hierbij te vervallen. Onze excuses voor het ongemak.

Meer informatie
Heeft u vragen, kijk dan op www.zbo-ccd.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:
- Factuur

Centrale Commissie Dierproeven

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Onze referentie
Aanvraagnummer
AVD8010020151

Factuur

Factuurdatum	25-02-2015
Vervaldatum	25-03-2015
Factuurnummer	2015701
Betreft	Factuur Aanvraag projectvergunning dierproeven

Omschrijving	**Bedrag**
Betaling leges projectvergunning dierproeven Betreft aanvraag AVD8010020151	€ 741,-

Wij verzoeken u het totaalbedrag vóór de gestelde vervaldatum over te maken op rekening NL28RBOS 056.99.96.066 onder vermelding van het factuurnummer en aanvraagnummer, ten name van Centrale Commissie Dierproeven, Postbus 20401, 2500 EK te 's Gravenhage.

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Centrale Commissie Dierproeven i.o.

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Onze referentie
AVD8010020151

Uw referentie

Bijlagen

Datum 19 februari 2015
Betreft Aanvullende informatie aanvraag projectvergunning

Geachte [redacted],

Op 11 Februari 2015 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "The role of stem cells and their derivatives during development, adult tissue homeostasis."

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

De in uw projectvoorstel en bijlagen dierproeven genoemde 'go no go momenten', zijn niet uitgewerkt. U wordt verzocht voor elk van de type dierproeven te beschrijven welke criteria zullen worden toegepast om tot een beslissing te komen over het wel of niet continueren van het onderzoek naar de functie van een specifiek gen.

In de bijlagen dierproeven beschrijft u een aantal verschillende methoden voor toediening van stoffen. U wordt verzocht aan te geven op basis van welke criteria voor welke toedieningsvorm gekozen gaat worden. Het cumulatief ongerief van de individuele dieren wordt mede bepaald door de gekozen methode van toediening. U wordt daarom verzocht aan te geven welk percentage van de dieren, naar verwachting, welke handelingen zullen ondergaan en in welke mate dit het cumulatief ongerief van de dieren zal beïnvloeden.

De DEC heeft in haar advies aangegeven dat er een overlap is tussen de huidige vergunningaanvraag en eerder van een positief advies voorziene DEC protocollen. Om een beeld te krijgen van de mate van overlap en het aantal proefdieren dat daarmee gemoeid is, is het wenselijk antwoord te krijgen op de volgende vragen:
-Hoeveel dieren zullen nog gebruikt worden op deze protocollen?
-Op welke in de vergunningaanvraag beschreven dierproeven heeft de overlap betrekking?
-In hoeverre is het noodzakelijk dat, daar waar er overlap is, de al eerder van een positief advies voorziene dierproeven en het huidige bij de CCD aangevraagde project naast elkaar uitgevoerd kunnen worden?

Opsturen informatie
De CCD zou graag uw aanvraag tijdens haar eerstvolgende vergadering behandelen. De CCD zou de gevraagde informatie derhalve uiterlijk woensdag 25 februari 2015 van u ontvangen. U kunt deze informatie aanleveren via NetFTP of per post. Indien u de informatie per post verstuurd, gebruik dan het bijgevoegde formulier.

Wanneer een beslissing
Zodra wij de aanvullende informatie hebben ontvangen, nemen wij uw aanvraag verder in behandeling. Als u goedkeuring krijgt op uw aanvraag, kunt u daarna beginnen met het project.

Meer informatie
Heeft u vragen, kijk dan op www.zbo-ccd.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 i.o.

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	

Hubrecht Institute

Developmental Biology
and Stem Cell Research

Royal Netherlands Academy of Arts and Sciences
University Medical Center Utrecht

Uppsalalaan 8
3584 CT Utrecht
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I www.hubrecht.eu

Centrale Commissie Dierproeven
Postbus 20401
2500EK Den Haag

Datum: Utrecht, 22 februari 2015.
Uw referentie: AVD8010020151

Geachte Leden van de CCD,

Deze brief schrijven wij U n.a.v. Uw brief d.d. 19 februari 2015. In deze brief verzocht U ons om de door U gestelde aanvullende vragen, welke nodig zijn om projectvergunning dierproeven (AVD8010020151) verder te kunnen beoordelen, te beantwoorden. Hierbij voldoen wij aan dit verzoek.

De in uw projectvoorstel en bijlagen dierproeven genoemde 'go no go momenten', zijn niet uitgewerkt. U wordt verzocht voor elk van de type dierproeven te beschrijven welke criteria zullen worden toegepast om tot een beslissing te komen over het wel of niet continueren van het onderzoek naar de functie van een specifiek gen.

Door de hele projectbeschrijving zijn op verschillende momenten go/no go momenten ingelast. Deze zijn inderdaad niet expliciet aangegeven als '*go/no-go*' maar bijvoorbeeld in termen van '*only if*', '*if required*', '*in case relevant*' en '*experiments wil only be carried out if*'. Daarnaast (maar dit is ook niet expliciet als een *go/no-go* aangegeven) houdt de gefaseerde uitvoering van experimenten impliciet ook verschillende go/no-go momenten in. Wij zullen de belangrijkste go/no-go beslismomenten in ons onderzoek hieronder nader toelichten:

1. Het onderzoek naar de functie van een bepaald gen begint altijd met uitgebreid literatuur onderzoek, overleg met binnen- en buitenlandse collega's en de analyse van en/of in bestaande cellijnen/organoiden, patiënten materiaal en reeds verzameld weefselmateriaal. Indien nog steeds relevant, dan zullen we vervolgens de rol van dit gen bestuderen in cellijnen en/of organoiden middels genetische modificatie van het betreffende gen. Vaak zijn onze wetenschappelijke vragen met deze zeer uitgebreide analyses beantwoord. Soms wordt er een interessant fenotype gevonden waarbij het noodzakelijk is om de rol van dit gen tijdens complexe processen (o.a. ontwikkeling, instandhouding en herstel van weefsels en ziekte) via proefdierstudies te bestuderen.
   De uitvoering zoals weergegeven in het schema in de bijlage houdt dus impliciet een aantal zeer belangrijke go/no-go momenten in als het gaat om de al of niet uitvoering van dierexperimenten. Zoals hierboven ook al aangegeven spelen de experimenten met de organoiden een zeer belangrijke rol bij de go/no-go beslissingen. Bijvoorbeeld voordat er besloten wordt een transgene/knockout muis te importeren of te genereren wordt in genetisch gemodificeerde organoids onderzocht of er met het gewenste genotype een fenotype wordt gevonden. Indien dit niet het geval is zal er van de import/het genereren van de betreffende genetisch gemodificeerde muis afgezien worden.
   Algemeen: Als dit mogelijk of relevant is zal er altijd een experiment met een organoid worden uitgevoerd op basis waarvan besloten wordt tot het al of niet uitvoeren van een dierexperiment.

2. Tijdens de gefaseerde uitvoering van veel van onze proefdierstudies zijn er vele go/no-go momenten. Deze zijn afhankelijk van vele factoren waaronder het type onderzoek, de vraagstelling, het orgaan, het type cel etc. Deze go/no-go momenten werden altijd uitgebreid beschreven in onze DEC aanvragen en zullen in de toekomst onderdeel zijn van al onze aanvragen voor proefdierstudies bij de IvD.
   Ter verduidelijking drie typische voorbeelden van dit soort go/no-go momenten in onze proefdierstudies.
   Het eerste betreft een experiment waarbij we de rol van een specifiek gen tijdens homeostase van de darm willen bestuderen. Hierbij gebruiken we het bekende Cre-LoxP systeem om het betreffende gen specifiek in de darmen uit te schakelen waarna we vervolgens op verschillende tijdstippen de consequentie van deze deletie op de darmen willen bestuderen. We verzoeken dan ook in onze aanvragen om de darmen, welke iedere 8 dagen bijna compleet vernieuwd worden, te analyseren op dag 1, 3, 5, 7 en 24 na de Cre inductie. Dag 1, 3, 5 en 7 om het proces in de tijd te volgen en dag 24 om te bepalen wat de consequentie is nadat de gehele darm 3 keer vernieuwd is. We beginnen altijd met de dag 7 tijdspunt. Indien we geen fenotype vinden op dag 7 dan zullen de eerdere tijdspunten niet geanalyseerd worden. Vinden we een fenotype waarvan we vermoeden dat er mogelijk ongerief gaat optreden op latere tijdstippen dan zullen we de dag 21 analyse niet uitvoeren.
   Studies naar de ontwikkeling van een bepaald fenotype omvatten vaak zowel een analyse in embryo's als in postnatale dieren. Bij de gefaseerde analyse van de embryonale stadia spelen dezelfde overwegingen als in het voorbeeld hierboven een rol. Op het moment dat er op P18 geen fenotype wordt gevonden wordt er afgezien van de analyse van de jongere stadia. Bovendien op het moment dat er tijdens de embryonale ontwikkeling een fenotype lijkt te ontstaan met risico op constitutioneel ongerief na de geboorte zal afgezien worden van de analyse van de postnatale stadia.
   Het uitzoeken van de optimale transplantatie condities met organoids wordt altijd gedaan in wild type muizen. Pas en ook alleen als er condities zijn gevonden die uitzicht bieden op een succesvolle transplantatie zal de overstap naar de transplantatie van organoids in de meer belastende ziektemodellen worden gemaakt.

In de bijlagen dierproeven beschrijft u een aantal verschillende methoden voor toediening van stoffen. U wordt verzocht aan te geven op basis van welke criteria voor welke toedieningsvorm gekozen gaat worden. Het cumulatief ongerief van de individuele dieren wordt mede bepaald door de gekozen methode van toediening. U wordt daarom verzocht aan te geven welk percentage van de dieren, naar verwachting, welke handelingen zullen ondergaan en in welke mate dit het cumulatief ongerief van de dieren zal beïnvloeden.

De verschillende toedieningswijzen zijn voor drie verschillende doelen (bijv. transgen inductie, aanbrengen of herstel van weefsel schade, toedienen van specifieke labels (bijv. BrdU)). Deze staan aangegeven bij al de verschillende 'type dierproeven' in de projectaanvraag. In deze verschillende 'type dierproeven' gelden voor de uiteindelijke keuze steeds dezelfde overwegingen. Deze keuze wordt eigenlijk in alle gevallen bepaald door het beoogde doel. Natuurlijk spelen de ongerief consequenties voor de dieren hierbij ook een belangrijke rol.

De keuze van de toedieningswijze wordt in een aantal gevallen bepaald door de plaats waar we het effect willen zien (bijv. orale toediening bij bestudering van de effecten specifiek in de slokdarm, maag of darmen (<1%); en subcutane toediening bij bestudering van de lokale effecten in de huid (<1%).

Hubrecht Institute

Developmental Biology
and Stem Cell Research

In meeste gevallen kiezen we voor een systemische toediening. In dat geval zullen we kiezen tussen toediening via voedsel/drinkwater (voordeel: weinig belastend, nadeel: onbekend wanneer en hoeveel opgenomen wordt (<1%)), via de onderhuidse implantatie van een pompje (voordeel: dosis is precies bekend en langdurige constante afgifte, nadeel: pomp moet onderhuids geplaatst worden middels een kleine operatie (onder narcose en anesthesie) (1%). Echter in meer dan 97% van de gevallen volstaat een kortdurende systemische toediening via 1 of een paar intra peritoneale injecties waarmee de relevante stoffen toegediend worden. De keuze van de toedieningswijze heeft dus slechts op max. 1% van de gevallen invloed op het cumulatief ongerief.

De DEC heeft in haar advies aangegeven dat er een overlap is tussen de huidige vergunningaanvraag en eerder van een positief advies voorziene DEC protocollen. Om een beeld te krijgen van de mate van overlap en het aantal proefdieren dat daarmee gemoeid is, is het wenselijk antwoord te krijgen op de volgende vragen: -Hoeveel dieren zullen nog gebruikt worden op deze protocollen?-Op welke in de vergunningaanvraag beschreven dierproeven heeft de overlap betrekking? -In hoeverre is het noodzakelijk dat, daar waar er overlap is, de al eerder van een positief advies voorziene dierproeven en het huidige bij de CCD aangevraagde project naast elkaar uitgevoerd kunnen worden?

Om de continuïteit van ons onderzoek te garanderen en omdat een aantal op dit moment lopende, goedgekeurde DEC protocollen allemaal in een verschillend stadium van uitvoering zijn is het onvermijdelijk dat er een overlap is tussen deze projectaanvraag en de lopende DEC protocollen. Na het verlenen van de projectvergunning door de CCD zullen al onze proefdieren en dierexperimenten formeel gaan vallen onder deze vergunning en zullen de dieren en de experimenten ‘afgeschreven’ worden van de beschrijvingen en aantallen in projectbeschrijving. Het enige is dat experimenten beschreven in de nog lopende DEC-protocollen niet (nogmaals) voorgelegd zullen worden aan de IvD. Dat zal natuurlijk wel gebeuren voor alle experimenten uit de projectaanvraag die nog niet beschreven staan in een nog lopend DEC-protocollen. Er is derhalve dan ook geen sprake van overlap c.q. het naast elkaar uitvoeren van experimenten beschreven in de DEC protocollen en in de projectvergunning. Er zullen hierover met de IvD heldere afspraken worden gemaakt.

Wij hopen U hiermee voldoende geïnformeerd te hebben en zien Uw reactie met belangstelling tegemoet.

Hoogachtend,

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

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Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

DEC KNAW
[redacted]
[redacted]
[redacted] Amsterdam

Centrale Commissie Dierproeven

Postbus 20401
2500 EK Den Haag
www.zbo-ccd.nl

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

info@zbo-ccd.nl

Onze referentie
AVD8010020151

Uw referentie

Bijlagen

Datum 02 maart 2015
Betreft Aanvullende informatie vergunningsaanvraag

Geachte [redacted]

Op 11 Februari 2015 hebben wij een aanvraag voor een projectvergunning dierproeven ontvangen waarover uw DEC advies heeft uitgebracht. Het gaat om het project "The role of stem cells and their derivatives during development, adult tissue homeostasis, diseases and regenerative medicine"

Welke informatie nog nodig
DE CCD heeft nog een vraag voor uw commissie. De aanvragers hebben aangeven dat de embryo's en neonaten eerst 10 minuten op ijswater geplaatst zullen worden voordat ze gedecapiteerd worden. Kunt u toelichten op welke wijze u het plaatsen van de embryo's en neonaten voor 10 minuten op ijswater (zonder direct contact) heeft meegewogen in uw advies? De CCD verzoekt u in elk geval toe te lichten in hoe verre u deze handeling noodzakelijk acht voor het bereiken van het doel en hoe u het door deze handeling veroorzaakte ongerief heeft meegewogen in de ongeriefsinschatting.

Opsturen informatie
De CCD zou de gevraagde informatie graag uiterlijk dinsdag 03 maart 2015 van u ontvangen.

Meer informatie
Heeft u vragen, kijk dan op www.zbo-ccd.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 i.o.

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	

KONINKLIJKE NEDERLANDSE
AKADEMIE VAN WETENSCHAPPEN

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag

Datum 3 maart 2015
Betreft Aanvullende informatie vergunningsaanvraag AVD8010020151

Geachte Leden van de CCD,

Deze brief schrijven wij n.a.v. uw brief d.d. 2 maart 2015. In deze brief vraagt u om aanvullende informatie over het advies dat de DEC-KNAW heeft uitgebracht over de aanvraag projectvergunning dierproeven (AVD8010020151). Hierbij voldoen wij aan dit verzoek.

Uw vraag is de volgende: De aanvragers hebben aangeven dat de embryo's en neonaten eerst 10 minuten op ijswater geplaatst zullen worden voordat ze gedecapiteerd worden. Kunt u toelichten op welke wijze u het plaatsen van de embryo's en neonaten voor 10 minuten op ijswater (zonder direct contact) heeft meegewogen in uw advies? De CCD verzoekt u in elk geval toe te lichten in hoe verre u deze handeling noodzakelijk acht voor het bereiken van het doel en hoe u het door deze handeling veroorzaakte ongerief heeft meegewogen in de ongeriefsinschatting.

Het verkrijgen van weefsel van muis embryo's en neonaten is een onmisbaar onderdeel voor het bereiken van de doelstellingen van het project. Het doden van embryo's en neonaten is daarom een noodzakelijke handeling.
De DEC heeft kennis genomen van de manier van doden van embryo's en neonaten zoals beschreven in de projectaanvraag. De commissie is bekend met de algemene overwegingen ten aanzien van het doden van muisembryo's en neonaten en de overwegingen van de indiener om te kiezen voor de in dit project beschreven methode.

De gangbare manier om juveniele en volwassen muizen te doden is via blootstelling aan CO_2/O_2 gevolgd door 100% CO_2. Het brein van embryo's en neonaten is niet zo exclusief afhankelijk van een oxidatief metabolisme zoals dat van juveniele en adulte dieren. Het doden door blootstelling aan CO_2/O_2 (anoxie) duurt daarom bij deze jonge dieren lang en gaat gepaard met meer ongerief in verband met het lang intact blijven van de hersenactiviteit. Deze methode is dan ook volgens de richtlijn 2010/63/EU bijlage IV niet toegestaan voor het doden van foetale en pasgeboren knaagdieren.
De combinatie hypothermie/decapitatie is een veel gebruikte en internationaal geaccepteerde methode voor het doden van muisembryo's en neonaten. Deze methode wordt binnen de KNAW en door vele nationale en internationale instellingen toegepast. De snelle afkoeling zorgt voor een snel verlies van hersenactiviteit/bewustzijn/pijnprikkels waarna de dieren worden gedood door decapitatie. De hersenen worden na de decapitatie direct ingevroren om opwarming van de hersenen en het eventueel weer terugkomen van de hersenactiviteit te voorkomen.

De DEC volgt de indiener in zijn overwegingen dat het koelen voorafgaand aan het doden het ongerief vermindert ten opzichte van het onverdoofd decapiteren van zeer jonge dieren en daarom gezien moet worden als een Verfijning in het kader van de 3V's.
Het ongerief ten gevolge van het doden van embryo's en neonaten door decapitatie na koelen wordt door de indiener ingeschat als licht en de DEC volgt deze inschatting. Het gebruik van deze methode heeft slechts een kleine bijdrage aan het cumulatieve ongerief op basis waarvan de DEC haar afweging heeft gemaakt.

Vertrouwelijke informatie
Gebruik door derden of
openbaarmaking is niet toegestaan.

Het Trippenhuis
[redacted]
[redacted] Amsterdam
Telefoon [redacted] . Fax [redacted]
[redacted]

Wij hopen u hiermee voldoende geïnformeerd te hebben en zien uw reactie met belangstelling tegemoet.

Met vriendelijke groeten, namens de DEC-KNAW

[redacted]

[redacted]

Vertrouwelijke informatie
Gebruik door derden of
openbaarmaking is niet toegestaan.

Het Trippenhuis
[redacted] Amsterdam
Telefoon [redacted] . Fax [redacted]

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Retouradres Postbus 20401 2500 EK Den Haag

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

Onze referentie
Aanvraagnummer AVD8010020151
Bijlagen: 4

KNAW
[redacted]
Postbus 19121
1000 GC AMSTERDAM

Datum: 5 maart 2015
Betreft: Beslissing Aanvraag projectvergunning Dierproeven

Geachte [redacted]

Op 11 februari 2015 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Uw aanvraag heeft betrekking op het project "The role of stem cells and their derivatives during development, adult tissue homeostasis, diseases and regenerative medicine" met aanvraagnummer AVD8010020151. Wij hebben uw aanvraag beoordeeld.

Besluit op aanvraag

Naar aanleiding van uw aanvraag delen wij u mede dat is besloten uw aanvraag voor de periode van 5 maart 2015 tot 1 maart 2020 **toe te wijzen** (gelet op artikel 10a lid 8 Wet op de dierproeven, hierna: Wod). Hierna kunt u lezen op grond van welke overwegingen wij tot deze beslissing zijn gekomen.

Het besluit wordt afgegeven onder de bepaling dat de bij dit besluit bijgesloten bijlagen deel uitmaken van de beschikking.

Procedure

De besluitvormingsprocedure is uitgevoerd overeenkomstig het bepaalde in paragraaf 3 van de Wod, paragraaf 2 van het Dierproevenbesluit 2014 en paragraaf 3 van de Dierproevenregeling 2014. En de overgangsregeling zoals die door de CCD op haar website is gepubliceerd.

Bij uw aanvraag heeft u een advies gevoegd van de dierexperimentencommissie DEC KNAW. Dit advies is opgesteld op 11 februari 2015. Overeenkomstig artikel 10a lid 3 van de Wod hebben wij dit advies betrokken bij de beoordeling van uw aanvraag. Wij kunnen ons vinden in de inhoud van het advies van de dierexperimentencommissie. In een zodanig geval kan worden volstaan met een verwijzing naar het advies, dat hier als herhaald en ingelast dient te worden beschouwd. Op grondslag van dit advies en op grondslag van de artikelen 9, 10, 10a2, 10a4, 10b, 10d tot en met 10h, 11, 13 en 13f van de Wod besluiten wij de aanvraag toe te kennen.

Meer informatie

Heeft u vragen, kijk dan op www.zbo-ccd.nl. Of neem contact met ons op via telefoonnummer: 0900 28 000 28 (10 ct/minuut) of per e-mail: ZBO-CCD@minez.nl.

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.

Het besluit geldt ook tijdens de bezwaarprocedure. 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.

Hoogachtend,

het Bestuur van de Centrale Commissie Dierproeven,

namens deze,

[redacted]

Prof. Dr. L. Hellebrekers
Voorzitter van de Centrale Commissie Dierproeven

Dit besluit is genomen met inachtneming van het Besluit mandaat, volmacht en machtiging van de Centrale Commissie Dierproeven CCD 2014 zoals het bestuur van de Centrale Commissie Dierproeven heeft vastgesteld op 19 december 2014, ref 2014-04 en is gepubliceerd in de Staatscourant van 2 januari 2015, Nr. 163

Bijlagen:

- Projectvergunning
- Aanvraag
- DEC Advies d.d. 11 februari 2015
- Niet Technische Samenvatting

Projectvergunning

gelet op artikel 10a van de Wet op de Dierproeven

Verleent de Centrale Commissie Dierproeven aan

Naam: KNAW
Adres: Postbus 19121
Postcode en plaats: 1000 GC AMSTERDAM
Deelnemersnummer: 80102

Deze projectvergunning is voor het tijdvak van 1 maart 2015 tot 1 maart 2020, voor het project "The role of stem cells and their derivatives during development, adult tissue homeostasis, diseases and regenerative medicine" met aanvraagnummer AVD8010020151, volgens advies van Dieren experimentencommissie DEC KNAW.

De verantwoordelijk onderzoeker is [redacted]
De aanvraag omvat de volgende bescheiden:
1 een aanvraagformulier projectvergunning dierproeven, ontvangen op 11 februari 2015
2 de bij het aanvraagformulier behorende bijlagen:
a projectvoorstel, zoals ontvangen per brief op 11 februari 2015;
b niet-technische samenvatting van het project, zoals ontvangen per e-mail op 11 februari 2015.
c het DEC advies d.d. 11 januari 2015, ontvangen op 11 februari 2015

Naam proef	Diersoort/ Stam	Totaal aantal dieren aangevraagd	Ongerief	Voorwaarden
Generation of novel (compound) GM mice and identification of phenotype's with constitional discomfort	Mus Musculus: -Wild type -Genetisch gemodificeerd	14.980 muizen	Donoren: mild Surrogaat: matig Genetisch gemodificeerd: mild	Niet van toepassing
Tissue, lineage and/or cell type specific gene (in)activation and (over)(mis)expression.	Mus Musculus: -Genetisch gemodificeerd	7.000 muizen	Embryo's: mild Neonaten: mild Volwassenen: 99% mild, 1% matig	Niet van toepassing
Lineage tracing and regeneration	Mus Musculus: -Genetisch gemodificeerd	7.000 muizen	Embryo's: mild Neonaten: mild Volwassenen: 75% mild, 25% matig	Niet van toepassing
Generation and analysis of disease models caused by deregulated stem or niche cells	Mus Musculus: -Genetisch gemodificeerd	4.000 muizen	Volwassenen: 99% mild, 1% matig	Niet van toepassing
Growth and genetically modification of organoids and (xeno) transplantation.	Mus Musculus: -Wild type -Genetisch gemodificeerd	1.600 muizen	matig	Niet van toepassing

Bijzondere voorwaarden

Op grond van artikel 10a1 lid 2 Wod zijn aan een projectvergunning voorwaarden te stellen

De vergunning wordt verleend onder de voorwaarde:
Daar waar er sprake is van overlap tussen de in deze vergunning vergunde dierproeven en eerder goedgekeurde DEC protocollen zullen de dieren en experimenten na het verlenen van de vergunning formeel onder deze vergunning gaan vallen, zoals u in uw brief van 22 februari 2015 ook heeft aangegeven. Hierdoor is er geen sprake meer van overlap.

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 13c 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 13c 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 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.

Beoordeling achteraf

Volgens artikel 10a1 derde lid van de wet worden projecten waarbij niet-menselijke primaten worden gebruikt, projecten die als ernstig ingedeelde dierproeven omvatten of een dierproef die leidt tot ernstige mate van pijn, lijden, angst of blijvende schade die waarschijnlijk langdurig zal zijn en niet kan worden verzacht, achteraf beoordeeld worden. In dit project worden dierproeven toegepast waarbij die vallen in de categorie ernstig volgens artikel 10b van de wet en wordt daarom voorzien van retrospectieve toetsing.

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* 80102 (Hubrecht Instituut-KNAW)
☐ Nee > *U kunt geen aanvraag doen*

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

Naam instelling of organisatie	KNAW
Naam van de portefeuillehouder of diens gemachtigde	[redacted]
KvK-nummer	5 4 6 6 7 0 8 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	
Postbus	Postbus 19121
Postcode en plaats	1000GC Amsterdam
IBAN	NL94DEUT0552026182
Tenaamstelling van het rekeningnummer	Hubrecht Instituut

1.4 Vul de gegevens in van de verantwoordelijke onderzoeker.

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

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

(Titel) Naam en voorletters	[redacted] ☑ Dhr. ☐ Mw.
Functie	Senior Scientist
Afdeling	[redacted]
Telefoonnummer	0 3 0 2 1 2 1 8 4 6
E-mailadres	[redacted]

4 Betaalgegevens

4.1 Om welk type aanvraag gaat het?

- [x] Nieuwe aanvraag Projectvergunning € 741,00 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.

- [x] Via een eenmalige incasso
- [ ] Na ontvangst van de factuur

5 Checklist bijlagen

5.1 Welke bijlagen stuurt u mee?

Verplicht

- [x] Projectvoorstel
- [x] Niet-technische samenvatting

Overige bijlagen, indien van toepassing

- [ ] Melding Machtiging
- [x] Flow chart experimenten

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	[redacted]
Plaats	Amsterdam
Datum	11-02-2015
Handtekening	[redacted]