		Inventaris Wob-verzoek W17-07								
			wordt verstrekt				**weigeringsgronden**			
nr.		**document NTS 2017840**	**reeds openbaar**	**niet**	**geheel**	**deels**	**10.1.c**	**10.2.e**	**10.2.g**	**11.1**
1		Aanvraagformulier				x		x		
2		NTS initieel			x					
3		Project proposal			x					
4		bijlage animal procedure 1			x					
5		bijlage animal procedure 2			x					
6		bijlage animal procedure 3			x					
7		bijlage animal procedure 4			x					
8		Ontvangstbevestiging				x		x		
9		acceptatiebrief				x		x		
10		mail vragen CCD				x		x		
11		vragen CCD aan vergunninghouder				x		x		
12		mail vragen CCD aan DEC				x		x		
13		mailwisseling over vragen				x		x		
14		reactie DEC op vragen				x		x		
15		DEC advies initieel				x		x		
16		DEC advies aangepast				x		x		
17		Project proposal definitief			x					
18		bijlage animal procedure 1 definitief			x					
19		bijlage animal procedure 2 definitief			x					
20		bijlage animal procedure 3 definitief			x					
21		bijlage animal procedure 4 definitief			x					
22		NTS definitief	x							
23		Advies CCD aan bestuur		x						x
24		Beschikking				x		x		

AVD 301002016840

24 JAN. 2017

Centrale Commissie Dierproeven

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.centralecommissiedierproeven.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*	30100
☐ Nee > *U kunt geen aanvraag doen*	

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

Naam instelling of organisatie	Stichting Het Nederlands Kanker Instituut – Antoni van Leeuwenhoek ziekenhuis	
Naam van de portefeuillehouder of diens gemachtigde	[redacted]	
KvK-nummer	40530817	
Straat en huisnummer	Plesmanlaan	121
Postbus	90203	
Postcode en plaats	1006 BE	Amsterdam
IBAN	NL71DEUT0626343534	
Tenaamstelling van het rekeningnummer	Stichting Het Nederlands Kanker Instituut – Antoni van Leeuwenhoek Ziekenhuis	

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.

1.4 Vul de gegevens in van de verantwoordelijke onderzoeker.

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

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

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

1.6 *(Optioneel)* Vul hier de gegevens in van de persoon die er verantwoordelijk voor is dat de uitvoering van het project in overeenstemming is met de projectvergunning.

(Titel) Naam en voorletters	[redacted]	☐ Dhr. ☒ Mw.
Functie	Instantie voor Dierenwelzijn	
Afdeling	[redacted]	
Telefoonnummer	[redacted]	
E-mailadres	[redacted]	

1.7 Is er voor deze projectaanvraag een gemachtigde?

☐ Ja > *Stuur dan het ingevulde formulier Melding Machtiging mee met deze aanvraag*

☒ Nee

2 Over uw aanvraag

2.1 Wat voor aanvraag doet u?

☒ Nieuwe aanvraag > Ga verder met vraag 3

☐ Wijziging op (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn

Vul uw vergunde projectnummer in en ga verder met vraag 2.2

☐ Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn

Vul uw vergunde projectnummer in en ga verder met vraag 2.3

2.2 Is dit een *wijziging* voor een project of dierproef waar al een vergunning voor verleend is?

☐ Ja > Beantwoord dan in het projectplan en de niet-technische samenvatting alleen de vragen waarop de wijziging betrekking heeft en onderteken het aanvraagformulier

☐ Nee > Ga verder met vraag 3

2.3 Is dit een *melding* voor een project of dierproef waar al een vergunning voor is verleend?

☐ Nee > Ga verder met vraag 3

☐ Ja > Geef hier onder een toelichting en ga verder met vraag 6

3 Over uw project

3.1 Wat is de geplande start- en einddatum van het project?	Startdatum	1 - 2 - 2017
	Einddatum	1 - 2 - 2022
3.2 Wat is de titel van het project?	Functional analysis of genes implicated in cancer	
3.3 Wat is de titel van de niet-technische samenvatting?	Functionale analyse van genen betrokken bij kanker	
3.4 Wat is de naam van de Dierexperimentencommissie (DEC) aan wie de instellingsvergunninghouder doorgaans haar projecten ter toetsing voorlegt?	Naam DEC	NKI
	Postadres	t.a.v. [redacted] Postbus 90203;1006 BE; Amsterdam
	E-mailadres	[redacted]

4 Betaalgegevens

4.1 Om welk type aanvraag gaat het?

☒ Nieuwe aanvraag Projectvergunning € 1684,- Lege

☐ Wijziging € Lege

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

☐ Via een eenmalige incasso

☒ Na ontvangst van de factuur

5 Checklist bijlagen

5.1 Welke bijlagen stuurt u mee?

Verplicht

☒ Projectvoorstel

☒ Niet-technische samenvatting

Overige bijlagen, indien van toepassing

☐ Melding Machtiging

☒

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 1.7). De ondergetekende verklaart:

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

Naam	[redacted]
Functie	Instantie voor Dierenwelzijn
Plaats	Amsterdam
Datum	20 - 1 - 2017
Handtekening	[redacted]

Format
Niet-technische samenvatting

- Dit format gebruikt u om uw niet-technische samenvatting te schrijven
- Meer informatie over de niet-technische samenvatting vindt u op de website www.centralecommissiedierproeven.nl.
- Of neem telefonisch contact op. (0900-2800028).

1 Algemene gegevens

1.1	Titel van het project	Functionale analyse van genen betrokken bij kanker
1.2	Looptijd van het project	5 jaar
1.3	Trefwoorden (maximaal 5)	Genfunctie, ontwikkeling, ontregeld, kanker, muismodel

2 Categorie van het project

2.1 In welke categorie valt het project.

U kunt meerdere mogelijkheden kiezen.

- x Fundamenteel onderzoek
- ☐ Translationeel of toegepast onderzoek
- ☐ Wettelijk vereist onderzoek of routinematige productie
- ☐ Onderzoek ter bescherming van het milieu in het belang van de gezondheid
- ☐ Onderzoek gericht op het behoud van de diersoort
- ☐ Hoger onderwijs of opleiding
- ☐ Forensisch onderzoek
- ☐ Instandhouding van kolonies van genetisch gemodificeerde dieren, niet gebruikt in andere dierproeven

3 Projectbeschrijving

3.1 Beschrijf de doelstellingen van het project (bv de wetenschappelijke vraagstelling of het wetenschappelijk en/of maatschappelijke belang)

Kanker is een van de belangrijkste doodsoorzaken in de westerse wereld en heeft grote gevolgen voor de maatschappij met betrekking tot individueel en algemeen welbevinden. Ook de gevolgen in economische zin zijn niet te onderschatten. De missie van ons instituut is het verminderen van kankerincidentie en het verbeteren van de behandeling van kankerpatiënten. Kanker is een verzameling van ziektes, die wordt gekenmerkt door een ontspoord gedrag van cellen waardoor de normale lichaamsfuncties worden verstoord en de patiënt kan komen te overlijden. Deze afwijkingen in het gedrag worden veroorzaakt doordat genen die betrokken zijn bij de regulatie van celdeling, cel beweging, celdood, etc. niet goed functioneren. Klinisch en fundamenteel onderzoek aan een breed scala van tumoren heeft een groot aantal genen geïdentificeerd die frequent ontregeld zijn in de ontwikkeling van kanker. Bij ontregeling kan het gaan om overactiviteit van genen dan wel

		om vermindering of zelfs verlies van gen activiteit. Met de toenemende effectiviteit van genoom analyse technieken zullen nog vele genen hieraan toegevoegd worden. Niet duidelijk is wat de rol van de ontregeling van deze genen is. Het doel van dit project is fundamentele kennis over de normale functie van deze genen. Deze kennis is essentieel voor het begrip van hun ontregeling voor de ontwikkeling van kanker en levert op termijn mogelijk nieuwe aanknopingspunten voor therapeutische interventie. Bovendien kan met deze kennis ingeschat worden wat de gevolgen van deze (gen) specifieke interventie kunnen zijn voor normale cellen en daarmee voor de patiënt. In dit project willen wij de normale functie van de in kanker ontregelde genen bestuderen in muismodellen. Hiertoe worden modificaties in deze genen geïntroduceerd in muizen en de gevolgen hiervan voor de normale ontwikkeling van deze muizen geanalyseerd. De muis is qua genetische opbouw vrijwel identiek aan de mens en is daarom een goed model voor deze studies.
3.2	Welke opbrengsten worden van dit project verwacht en hoe dragen deze bij aan het wetenschappelijke en/of maatschappelijke belang?	Wij verwachten dat we met dit project een schat aan fundamentele kennis opdoen over de normale functie van, in kanker frequent ontregelde, genen in de normale ontwikkeling van de muis en daarmee in de ontwikkeling van de mens. Deze kennis zal ons in staat stellen de betekenis van hun ontregeling voor de ontwikkeling van kanker beter te begrijpen. Bovendien zullen de effecten van eventuele therapeutische interventies gericht op de functie van deze genen op gezonde cellen en daarmee de patiënt beter ingeschat kunnen worden. De resultaten van dit project zullen leidend zijn voor het opstellen van vervolgstudies waarin de rol van de ontregelde genen in de ontwikkeling van kanker op zichzelf en in de effectiviteit van therapeutische interventie bestudeerd zal worden. Voor de dierexperimenten nodig in deze vervolgstudies zullen separate CCD licenties aangevraagd worden.
3.3	Welke diersoorten en geschatte aantallen zullen worden gebruikt?	Diersoort: Muis (mus Musculus) Geschat aantal: 2050+2350+9000+2880 = 16280
3.4	Wat zijn bij dit project de verwachte negatieve gevolgen voor het welzijn van de proefdieren?	-In dit project worden genetisch gemodificeerde dieren fenotypisch gekarakteriseerd. De dieren ondervinden hiervan geen ongerief behalve wanneer de genetische modificatie op zichzelf tot ongerief leidt. De aard en het niveau van dit ongerief is niet te voorzien, maar door de frequente monitoring als onderdeel van de verzorging zal het ongerief beperkt blijven tot matig. -In dit project worden geen chirurgische ingrepen uitgevoerd behalve in een zeer klein aantal dieren waarbij de genetische modificatie later in een deel van het lichaam wordt geïntroduceerd. Het ongerief zal hierbij niet hoger zijn dan matig. -Een zeer klein deel van de dieren zal betrokken zijn bij de studies van genen in weefselherstel. Deze dieren kunnen matig ongerief ondervinden.
3.5	Hoe worden de dierproeven in het project ingedeeld naar de verwachte ernst?	Licht ongerief in ca: 11825 (73%) Matig ongerief in ca: 4455 (27%)
3.6	Wat is de bestemming van de dieren na afloop?	De dieren worden gedood en de weefsels worden gebruikt in het kader van de doelstellingen van het onderzoek.

4 Drie V's

4.1	**Vervanging** Geef aan waarom het gebruik van dieren nodig is voor de beschreven doelstelling en waarom proefdiervrije alternatieven niet gebruikt kunnen worden.	Bij het ontstaan van kanker zijn een groot aantal genen ontregeld. Met het vooraf verrichten van uitgebreid pathologisch en 'in vitro' onderzoek selecteren we die genen waarvan de ontregeling waarschijnlijk kanker veroorzaakt dan wel de progressie ervan. In dit project willen we de normale functie van deze genen bestuderen in de ontwikkeling van een compleet/intact dier. Er zijn geen alternatieven hiervoor.
4.2	**Vermindering** Leg uit hoe kan worden verzekerd dat een zo gering mogelijk aantal dieren wordt gebruikt.	De ontwikkeling van de muis is tot in detail bekend en de analyse van eventuele afwijkingen hiervan in genetisch gemodificeerde dieren behoeft geen grote aantallen experimentele en controle dieren. Daarom blijft de groepsgrootte in iedere studie zeer gering.
4.3	**Verfijning** Verklaar de keuze voor de diersoort(en). Verklaar waarom de gekozen diermodel(len) de meest verfijnde zijn, gelet op de doelstellingen van het project.	De keuze voor de muis als proefdiermodel komt voort uit de volgende overwegingen: (1) De muis vertoont qua orgaanstructuur en genetische opbouw grote overeenkomsten met de mens. (2) Voor dit onderzoek belangrijke moleculair biologische technieken kunnen alleen in deze diersoort efficiënt worden uitgevoerd. (3) Er zijn veel verschillende stammen beschikbaar. (4) Het is een goed toegankelijk proefdier dat goed te houden en te hanteren is.
	Vermeld welke algemene maatregelen genomen worden om de negatieve (schadelijke) gevolgen voor het welzijn van de proefdieren zo beperkt mogelijk te houden.	De handelingen met de dieren worden enkel uitgevoerd door ervaren, gekwalificeerd personeel. Alle chirurgische handelingen die kunnen leiden tot ongerief of pijn worden onder adequate anesthesie en analgesie uitgevoerd. Er vindt nauwgezette postoperatieve monitoring plaats van de muizen en humane eindpunten worden toegepast. Alle beschikbare middelen om ongerief te vermijden of verminderen worden toegepast.

5 In te vullen door de CCD

Publicatie datum	
Beoordeling achteraf	
Andere opmerkingen	

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.centralecommissiedierproeven.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'.	30100
1.2	Provide the name of the licenced establishment.	Stichting Het Nederlands Kanker Instituut – Antoni van Leeuwenhoek ziekenhuis
1.3	Provide the title of the project.	Functional analysis of genes implicated in cancer

2 Categories

2.1	Please tick each of the following boxes that applies to your project.	x Basic research
		☐ Translational or applied research
		☐ Regulatory use or routine production
		☐ Research into environmental protection in the interest of human or
		☐ 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.

Cancer is caused by malfunctioning of genes regulating cellular processes e.g. cell division, differentiation

and migration. Normally these processes are properly controlled by adequate integration of gene activities in a complex network of signalling pathways. Changes in activity of these genes by mutation, under- or overexpression can result in an imbalanced pathway network leading to aberrant cell behaviour, e.g. uncontrolled proliferation, mobility, and ultimately to cancer. Notably, in tumors of distinct origin often the same pathways are affected by changes in different genes functional in these pathways.

Cancer research over the last decades comprised detailed genomic analyses of tumors from patients and animal models and large numbers of 'in vitro' and 'in vivo' screening approaches. This research has resulted in the identification of a large number of genes and genetic elements controlling them implicated in many different cancer types. However, since genomic analyses often occur at end stage tumor material the identification of these genes as such provides an essential but limited contribution to our understanding how they are involved in the tumorigenic process. For this, thorough functional study of these genes and genetic elements involving both 'in vitro' and 'in vivo' (animal) experiments in normal and oncogenic conditions is required. This yields fundamental knowledge necessary for understanding their possible role in tumor onset, development and progression, which is crucial for translational application in diagnostics and development of therapeutic intervention modalities.

The list of identified cancer genes to date is far from complete and with the still exponential growing potential of genome sequencing, screening and tagging technologies many new genes implicated in cancer development will be identified in the years to come. In this project we will select candidate genes and genetic elements on the basis of published data, e.g. cancer genomic databases from human and other species, and of data from in vitro and in vivo screening and tagging experiments. We want to acquire fundamental knowledge about the normal physiological function of (combination of) these genes and genetic elements by studying the phenotypic effects of controlled (expression) modification at the level of whole animals and at the cellular level, i.e. in GGO mice and cell cultures derived from them, respectively. This will be done in mice carrying the genetic modification in all cells (germ line modifications) or in mice in which the genetic modification is introduced in a tissue and/or temporal specific fashion (conditional modifications). In the latter situation we will also make use of reporter systems that enable to follow the modified cells in the course of development. Already available mouse strains carrying alleles with appropriate modifications will be imported. New strains will be generated in our institute under a separate license.

In general, the functional characterization of selected genes will initially entail the following aspects: viability of germ line modification, Mendelian transmission of the modification, phenotyping of the modified mice, comparison of their behaviour with wild type control mice and thorough molecular and histo-pathological analysis at different ages. In addition, we will derive cell cultures from modified mice to study the effects of the changed gene expression at the molecular and cellular level. The results of these analyses will be leading in the choice from which tissues from the modified mice cell cultures will be derived.

In case germ line modification of selected genes is not viable or certain strong phenotypes preclude the detailed analysis of other, more subtle phenotypes, we will make use of conditional technology enabling temporal and tissue specific gene modification. Examples of early embryo lethality of germ line knockout alleles (see references), which show clear (cancer) phenotypes when alleles are inactivated at adulthood and/or in specific tissues unnoticed in studies of embryo development: pRb, PTEN, APC, Brca1 and BRCA2 (see references below).

References
- Clarke AR, Maandag ER, van Roon M, van der Lugt NM, van der Valk M, Hooper ML, Berns A, te Riele H. Nature 1992, 359: 328-330, Requirement for a functional Rb-1 gene in murine development.
- Di Cristofano A, Pesce B, Cordon-Cardo C, Pandolfi PP. Nat Genet. 1998,19: 348-355, Pten is essential for embryonic development and tumour suppression.
- Oshima M, Oshima H, Kitagawa K, Kobayashi M, Itakura C, Taketo M.
Proc Natl Acad Sci U S A. 1995, 9;92: 4482-4486, Loss of Apc heterozygosity and abnormal tissue building in nascent intestinal polyps in mice carrying a truncated Apc gene.
-Hakem R, de la Pompa JL, Mak TW. J Mammary Gland Biol Neoplasia. 1998 3:431-445, Developmental studies of Brca1 and Brca2 knock-out mice.

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?

The objective of this project is to acquire fundamental knowledge about the normal, physiological function and their role in cellular signaling pathways of specific genes of which the activity is frequently deregulated in cancer development. This knowledge is essential to understand how this deregulation could affect the cross talk with other genetic lesions leading to tumorigenesis. Furthermore, these studies are necessary to predict the consequences of eventual therapeutic intervention strategies specifically targeting the activity of these genes. Moreover, this knowledge will provide essential leads for subsequent research projects for which eventually separate CCD license applications will be assembled. Under this project we will analyze the role of these genes selected on the basis of their data based relevance for tumor development, each in a separate study. In all these gene specific studies we will follow the same routine procedures: phenotyping of the modified mice, thorough histo-pathological and molecular analyses at different ages and characterization of cell cultures derived from these mice.
The results of these studies together with existing data will be leading in designing subsequent 'in vivo' experiments addressing the oncogenic effects of (combinations of) deregulated genes thereby validating their role in human cancers and providing (animal) models for testing therapeutic intervention modalities. These subsequent experiments will be described in additional license applications.
We think we can achieve this objective since our institute holds both the scientific knowledge and technological expertise for these studies: a modern state of the art animal facility including internationally recognized transgenic and imaging facilities embedded in the NWO Roadmap funded MCCA, a well trained staff taking care of animal husbandry, a dedicated animal pathology department with two mouse pathologists supported by a well-equipped pathology lab with all histological technologies available. In addition, all research departments within our institute have access to cell culture facilities, sophisticated digital microscopy (confocal, live-imaging, etc.) and flow cytometry facilities.

3.3 Relevance

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

Scientific: To understand the role of genes identified in large genomic and screening/tagging approaches in the process of cancer onset and progression it is essential to know what the functional role of these genes is in normal development and physiology. This knowledge is also crucial to set up therapeutic

intervention strategies targeting the cellular pathways in which these genes are active.
Social: Cancer has a major negative impact on health / wellbeing of many people. Moreover, the morbidity also has considerable negative economic consequences. There is an urgent need to improve existing treatment strategies since many of them have serious side effects, which limit their full application, since they are often based on targeting (generic) cellular processes also essential for normal healthy cells. Cancer research over the last decades has resulted in the identification of many genes causally implicated in cancer development, thereby providing many possible specific targets for new therapy modalities. The knowledge of the normal function of these genes is essential for the development of targeted therapeutic intervention strategies in evaluating their effectiveness and serious adverse effects. As an illustration of the relevance of these studies see below a short list of publications describing the phenotype of mice carrying modifications in cancer implicated genes and the possible adverse effects as a consequence of therapeutic intervention targeting the their activity.

-Mikkers H, Nawijn M, Allen J, Brouwers C, Verhoeven E, Jonkers J, Berns A. 2004, Mol Cell Biol. 24: 6104-6115, Mice deficient for all PIM kinases display reduced body size and impaired responses to hematopoietic growth factors.
-An N, Kraft AS, Kang Y. 2013, J Hematol Oncol. 6:12, Abnormal hematopoietic phenotypes in Pim kinase triple knockout mice.
-Zhang HW, Ding J, Jin JL, Guo J, Liu JN, Karaplis A, Goltzman D, Miao D. 2010, J Bone Miner Res. 25: 640-652. Defects in mesenchymal stem cell self-renewal and cell fate determination lead to an osteopenic phenotype in Bmi-1 null mice.
-Gu M, Shen L, Bai L, Gao J, Marshall C, Wu T, Ding J, Miao D, Xiao M. 2014, Age 36: 129-139. Heterozygous knockout of the Bmi-1 gene causes an early onset of phenotypes associated with brain aging.

3.4 Research strategy

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

In this project we want to characterize the normal function of cancer related genes in development and physiology at the level of a complete animal. As such the project comprises of many different smaller studies each focusing on a specific gene, gene family or element or set of complementing genes. These studies share the relationship with and relevance for cancer and in practice they follow the same course of straightforward experiments. For a schematic overview of the route each study will follow see flow chart.
Each study starts with the selection of a particular cancer related gene on the basis of existing data indicating their relevance for (human) cancer. For each gene project the following steps will be carried out:

- First we will search literature and depositories for already available mouse strains carrying the gene modification of interest. If available at other laboratories or depositories we will import these mice, if not we will generate the mouse strains in the MCCA facility in our institute and perform the welfare assessment of newly generated GM mice according to the European guidelines. The procedures to generate GM themselves are described in a separate license application from our institute (‘*Generation and cryopreservation of genetically modified mouse strains’*).
  According to the ‘*Herziene Wet op de Dierproeven: het genereren, fokken, genotyperen, monitoren en huisvesten van genetisch gewijzigde dieren’* (Versie: oktober 2016), the welfare assessment of newly

generated genetically modified mouse strains does not require a CCD license upfront, but a license is required for newly genetically modified mouse lines when they exhibit an affected phenotype in their welfare assessment. Beforehand we cannot predict whether the modification of cancer related genes selected for further investigation will lead to an affected phenotype. However, we estimate that up to 10% of the newly generated modified mouse lines might exhibit an affected phenotype and we only apply for a CCD license for this number of mice (appendix 1). The estimation of 10% is based on our own experience and on published data from large phenotyping consortia (Dickinson et al. 2016, Nature 537: 508-514, High-throughput discovery of novel developmental phenotypes).

- In case germ line modifications preclude the analysis of subtle or cell type specific functions at later stages, e.g. in case of embryonic lethality, we will introduce the gene modifications in a temporally controlled and/or tissue/cell type specific fashion. For this we will make use of conditional targeting technology. This approach requires the generation of conditional alleles of the genes of interest and of mouse strains expressing various 'switch' genes enabling controlled activation of gene modifications. Alternatively, 'switch' genes may be introduced somatically (e.g. using viral vectors). These 'switch' genes include sequence specific recombinases (e.g. Cre and Flp) or gene editing systems (e.g. Crispr/Cas). In addition, reporter systems enabling the tracing of cells carrying the modified gene of interest will be incorporated, thereby refining the read out of the experiments. Mouse strains carrying 'switch' alleles and reporter alleles will be referred to as 'tool box' strains. Mouse strains involved in these analyses, i.e. those carrying conditional alleles for the genes of interest, and the 'tool box' strains will either be imported or generated in our transgenic facility. In the latter case, experiments validating their effectiveness will be executed. Validation of these strains and additional technology enabling conditional modification will be described in appendix 2.
- To study the effects of gene modification in all cells of the mouse we will set up cohorts of germ line modified mice and appropriate controls. First we will monitor viability and Mendelian transmission. In case the germ line modification is viable we will follow the modified mice over time and score for aberrant development and behavior. In addition we will perform a thorough molecular and histo-pathological analysis at different ages. In cases of genes that have similar (redundancy, e.g. gene families) or complementary molecular activities we might need to combine modification of gene family members or complementing genes in order to uncover their physiological function. If germ line modification is not viable we will first characterize the phenotype at different stages of gestation. For this we will set up breeding pairs to produce pregnant females carrying modified and control foetuses. In case we want to study gene function at later stages and in specific tissues/cell types we will take the conditional approach and we combine the necessary genetic components to generate the experimental and control cohorts of mice. Decision on timing and tissue/cell type specificity will also be based on the tumor type in which the gene of interest was found to be implicated in addition to their expression profile and other data. After inducing the gene modifications mice will be followed over time and scored for aberrant development and behavior. In addition we will perform a thorough molecular and histo-pathological analysis. In addition to these analyses we will derive cell cultures from modified mice to study the effects of the genetic modification on a cellular and molecular level under strictly defined conditions.

  All these phenotyping and molecular characterization experiments will be described in appendix 3.
- The phenotypic analysis described in appendix 3 will be done on mice kept under standard conditions, not treated in any way. However, existing data from other sources and the results of the analyses described in appendix 3 may indicate that for some genes their function can only be uncovered when

the modified mice or tissues/cells derived from them are exposed to challenging conditions. This might especially be the case when they have a role in stem cell performance supporting tissue homeostasis and regeneration, which as such is also relevant for tumor maintenance. Appendix 4 describes short term pilot experiments involving challenging conditions to obtain the leads for separate, additional license applications that address on a broad scale and in greater depth the function of these genes under specific conditions.

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

The procedures for the generation of genetically modified mouse strains are covered by a separate protocol from the transgenic facility of our institute. Appendix 1 describes the initial welfare assessment of newly generated mouse strains.
The required modifications and technology for conditional induction of genetic modifications will be validated as described in appendix 2 before setting up cohorts for phenotypic characterization of mouse strains carrying modified cancer related genes.
This phenotypic analysis is described in appendix 3 and requires the set up of experimental and control cohorts by conventional breeding procedures. When mice carry conditional modifications, mice will undergo treatments activating the genetic modification of interest. Mice will be closely monitored over time. At increasing ages mice will be sacrificed and total necropsy will be performed. All tissues will be carefully analyzed by molecular and histo-pathological assays. For ' vitro' studies we will also derive cell cultures from the genetically modified mouse strains.
In a minority of cases genetically modified mice or tissues/cells derived from them will be studied in vivo under challenging conditions as described in appendix 4. These experiments address the role of genes primarily involved in tissue regeneration and include transplantation procedures.

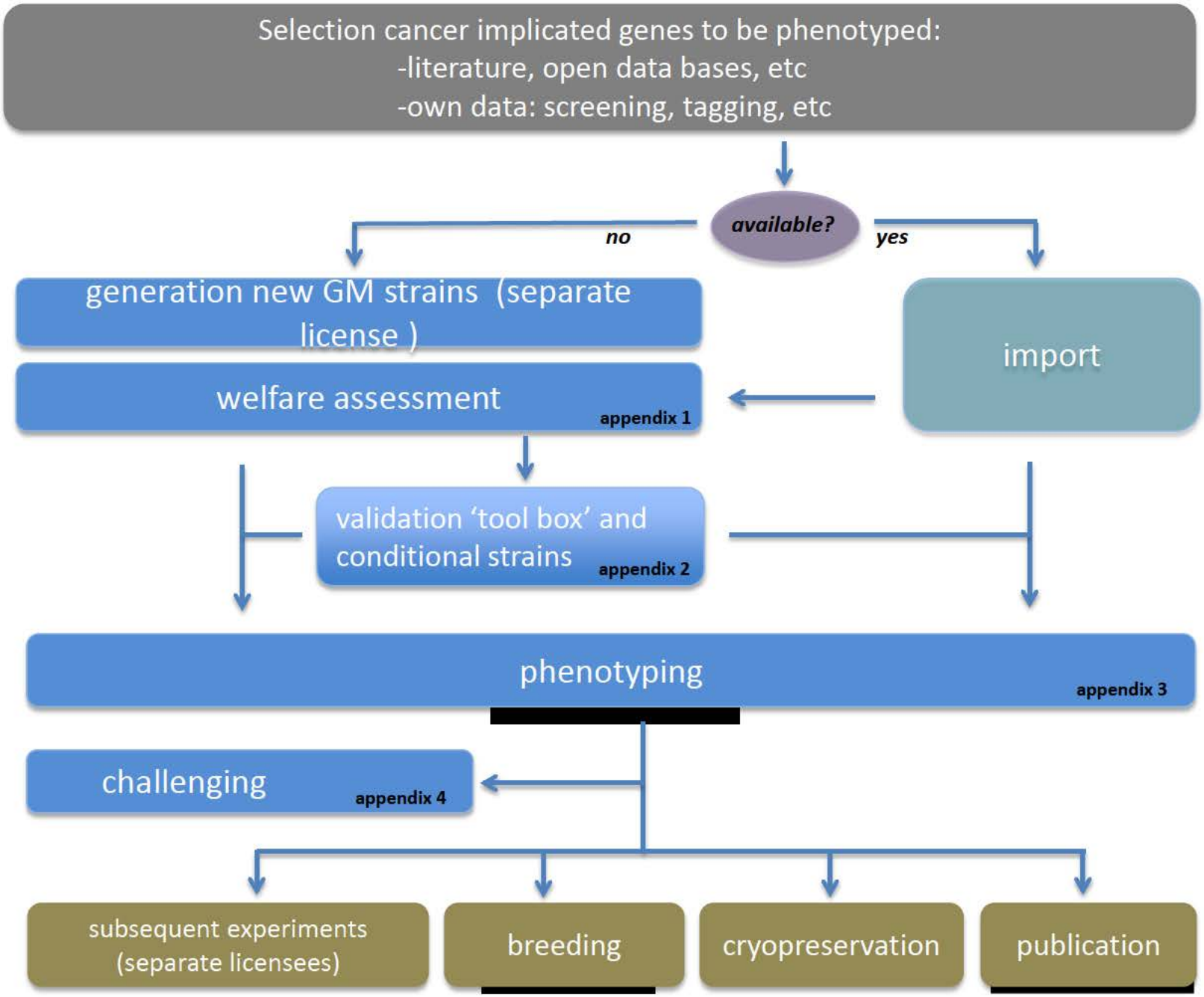
3.4.3 Describe the coherence between the different components and the different steps of the project. If applicable, describe the milestones and selection points.

The flow chart presents an overview of how the project is organized. The coherence of the project is reflected by the fact that each study will take the same approach and consists of a preparation phase and an analytical phase in which the same experimental procedures are followed. In the preparation phase for each study the mouse strains carrying the genetic modification of interest are acquired, their welfare assessed and validated in case of 'tool box' strains (appendices 1 and 2). The analytical phase (appendix 3 and 4) for each separate study follows the same procedure: setting up appropriate cohorts, activation of the modifications if needed, phenotyping at different ages/stages, thorough molecular and histo-pathological characterization and cell culture derivation for ex vivo experiments (appendix 3). In a limited number of these studies the analysis phase will include the characterization of modified mice under challenging conditions (appendix 4). In the analytical phase also cell cultures will be derived from the genetically modified mice.

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	Welfare assessment of new genetically modified mice
2	Validation of conditional genetic modification technology
3	Phenotyping of mice carrying germ line or temporal and/or tissue specific genetic modifications
4	Functional analysis of genetic modifications in mice under challenging conditions

5	
6	
7	
8	
9	
10	


Selection cancer implicated genes to be phenotyped:
-literature, open data bases, etc
-own data: screening, tagging, etc
available?
no
yes
generation new GM strains (separate license)
import
welfare assessment
appendix 1
validation 'tool box' and conditional strains
appendix 2
phenotyping
appendix 3
challenging
appendix 4
subsequent experiments (separate licensees)
breeding
cryopreservation
publication

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.centralecommissiedierproeven.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'.	30100
1.2	Provide the name of the licenced establishment.	Stichting Het Nederlands Kanker Instituut – Antoni van Leeuwenhoek ziekenhuis

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	General welfare assessment of newly generated genetically modified (GM) mice

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 modified (GM) mice will take place via 1) classic transgenesis with DNA injection into zygotes, 2) genome editing technologies (e.g. CRISPR/Cas) in pre-implantation embryo's or 3) injection of genetically modified embryonic stem cells into blastocysts. These procedures will be performed under a separate protocol from our transgenics facility. Basic welfare assessment for the novel (compound) mouse models, for 2 breeding cycles from F2 onwards, is performed according to the guidelines of the new EU directive. Some combination lines will be obtained by conventional crossing of generated lines. New combination lines will be screened for two generations and monitored for spontaneous phenotypic abnormalities.

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

1) Tissue sampling of pups for genotyping and identification:

a. Toe clipping is performed at 5-7 days after birth OR

b. Ear clipping is performed after weaning. In order to improve the precision of the earmarks this is done under anesthesia according to SOP ('pain relief and anesthesia') from the Animal Facility.

The choice between toe- or ear-clip is dependent on the requirements or stage of breeding, as not always

all mice need to be genotyped shortly after birth.
In rare cases genotyping has to be repeated using an additional biopsy; this will be done by tail clipping under anesthesia according to SOP ('pain relief and anesthesia') from the Animal Facility.

2) Welfare assessment:
Mice are monitored daily as part of the standard care by the Animal Facility. In addition the mice will be checked frequently by the researchers for the several parameters as described in the Directive 2010/63/EU: corrigendum of 24 January 2013. The parameters are: appearance, relative size, behavior and posture, growth, coat condition, fertility and mortality figures, and clinical signs. In case of any discomfort more than mild the frequency will be increased.

3) Animals are killed according to SOP ('euthanasia of mice') from the animals facility for instance because they do not have the right genotype or display unacceptable suffering.

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. All techniques are state-of-the-art and are proven effective in modulating inducible gene modification and characterizing GM mice with minimal numbers of animals.

B. The animals

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

Mus musculus: Wild-type and GM mice. Wild-type mice are in part obtained from registered commercial companies; all other mice are obtained from internal breeding programs. Our institute is a license holder with NVWA.
Numbers: Based on our experience of the last 5 years, for the creation of a new GM mouse line we use on average up to 150 mice (according to the "Besluit Biotechnologie"). However, as the generation of the genetically modified strains per se will be performed under a different protocol from our transgenics facility (MCCA), we do not include these numbers in this current protocol.
The founder generations (F0) obtained from the transgenics facility consist on average of 30 animals per GM line.
For each germ line modification typically three independent founders are selected and initially bred to wild-type (e.g. FVB or B6) mice to create an F1. We will aim to generate substantial numbers of homozygous mice in the next generation (F2). Generations F2 to F4 will undergo an initial welfare assessment. The F4 is included in order to assess the full reproductive cycle over two generations, including gestation in homozygous parents (which does not usually apply to the F2). To have at least 7 males and 7 females for welfare assessment of each generation, we assume at least three litters (average size 7 pups) per generation will need to be generated. Assuming 30 mice for the F0, 30 for the F1 and F2 (to obtain sufficient numbers of male and female homozygous F2 mice), this yields 30 + 3x10 + 3x10 + 3x21 + 3x21 = 222 mice per genetic modification. Based on our experience from the last decade we expect to generate 50 germ line modified mouse lines for which we will need 50 x 222 = 11100 mice for welfare assessment.
For each conditional modification typically 2 independent founders will be selected. For welfare assessment we will need 30 + 2x10 + 2x10 + 2x21 + 2x21 = 154 mice per modification. Based on our experience from the last decade we expect to generate 25 conditionally modified mouse lines for which we will need 25 x 154 = 3850 mice for welfare assessment.
For each modified mouse line instrumental in the activation and/or tracing of conditional genetic

modifications ('tool box' mouse lines) 3 independent founders will be selected. For welfare assessment we will need 30 + 3x10 + 3x10 + 3x21 + 3x21 = 222 mice per modification. Based on our experience from the last decade we expect to generate 25 mouse lines instrumental in conditional genetic modification for which we will need 25 x 222 = 5550 mice for welfare assessment. In total we will need 20500 (11100 + 3850 + 5550) mice for the welfare assessment of 100 new genetically modified mouse lines. However, only for the lines that show an affected phenotype a CCD license is required. We cannot foresee of which lines the phenotype will be affected but we estimate that of up to 10% of the lines this might be the case. This figure is based on our own experience and published data from large phenotyping consortia (Dickinson et al. 2016, Nature 537: 508-514, High-throughput discovery of novel developmental phenotypes). Therefore, we need 0,10 x 20500 = 2050 mice licensed in this appendix.

C. Re-use

Will the animals be re-used?

x No, continue with question D.

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

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

xNo

☐ 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.

- Generation of a new (compound) GM line is carefully considered in advance and should in principle yield new information that can only be obtained by studying the effects of the change of gene activity in a complete animal. In addition, the precise genetic modification should be backed up by solid data and evidence, which can be obtained from a variety of sources, such as genetic screens, in vitro experiments or clinical (large-scale) patient data.
- Before a new GM mouse line is created, we first extensively scan the literature and public resources (EMMA/Infrafrontier, JAX, KOMP and others) for already available GM strains of the same gene or locus. Duplicate mouse strains are not produced.
- The publicly available resource (www.infrafrontier.eu) of genetically modified embryonic stem cells that our transgenics facility has created allows for the generation of compound GM mice without the need for subsequent breeding. This reduces the number of mice obtained by breeding efforts as all modified alleles are already present in the embryonic stem cells (Huijbers et al., Nat Protoc. 2015 Nov;10(11):1755-85. doi: 10.1038).
- The genetic modification will be performed as much as possible in the desired genetic background. This can be achieved by using gene editing technology in pre-implantation embryo's and ES cells from the relevant background. As a result the differences in genetic background of the experimental mice and control mice will be as small as possible thereby increasing the significance and reproducibility of the welfare assessment data.

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

1) All mice are monitored daily for signs of discomfort.

2) There are no negative environmental effects; all mice are housed in controlled environments in

individually ventilated cages (IVC). Mice get additional cage enrichment (for instance a mouse home and paper tissues) when signs of distress are observed.

Repetition and duplication

E. Repetition

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

n.a.

Accommodation and care

F. Accommodation and care

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

x No

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

G. Location where the animals procedures are performed

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

x No > Continue with question H.

☐ Yes > Describe this establishment.

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

Classification of discomfort / humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

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

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

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

Anesthesia will be applied during ear or tail clipping according to SOP ('pain relief and anesthesia') from the Animal Facility.

I. Other aspects compromising the welfare of the animals

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

The purpose of this appendix is to assess the welfare of mice carrying (conditional) modification in genes of unknown function. We cannot exclude the occurrence of adverse effects in some of the generated (compound) modified mouse strains. However, based on our 30 years' experience in this type of studies we expect that less than of 10% of the animals the phenotype will be affected and half of these might

suffer unexpected discomfort. The nature of the adverse effects and level of discomfort is unpredictable. Rarely, mice are born (the F0 founders) with elephant teeth or showing a severely retarded development, but this also occurs during normal breeding.
In all cases mentioned above the affected animals will be killed immediately in order to limit the discomfort level to moderate.

Explain why these effects may emerge.

In addition to the developmental and physiological consequences of the gene modifications for the handlings of the embryo's or ES cells (e.g. micro-injection or modification per se of ES cells) during the modification procedure might perhaps cause improper development in exceptional cases that emerge in the F0 founders.

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

Mice are monitored daily as part of the standard care by the Animal Facility. In addition the mice will be checked frequently by the researchers for the several parameters as described in the Directive 2010/63/EU: corrigendum of 24 January 2013. The parameters are: appearance, relative size, behavior and posture, growth, coat condition, fertility and mortality figures, and clinical signs. In case of any discomfort more than mild the frequency will be increased.
When discomfort reaches the level of moderate, mice will be euthanized.
Action will be taken on the same day if unexpected adverse effects 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.

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

In case mice are assessed as having a spontaneous discomfort level higher than moderate due to the genetic modification(s) they will not be continued and culled.

Indicate the likely incidence.

Based on our experience and published data (Dickinson et al. 2016, Nature 537: 508-514) only a small fraction (<5%) of genetically modified mouse strains might have spontaneous discomfort higher than mild.

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').

-GM mice, during welfare assessment: mild (or less) 90%, moderate: <10%
- Mice undergoing ear or tail clips: mild

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

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

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.

x Yes

Appendix
Description animal procedures

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- Or contact us by phone (0900-2800028).

1 General information

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1.2	Provide the name of the licenced establishment.	Stichting Het Nederlands Kanker Instituut – Antoni van Leeuwenhoek ziekenhuis

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
2	Validation of conditional genetic modification technology

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.

In case germ line modifications preclude the analysis of subtle or cell type specific functions at later stages, e.g. in case of embryonic lethality, we will introduce the gene modifications in a temporally controlled and/or tissue/cell specific fashion. For this we will make use of conditional technology. This approach requires the generation of conditional alleles of the genes of interest and of mouse strains expressing various constitutive or inducible 'switch' genes (e.g. recombinases such as Cre, Flp) that activate the genetic modification in conditional genes. Alternative approaches that introduce the 'switch' genes somatically will be involved as well. For example, the use of viral vectors carrying cell type specific promoters driving 'switch' gene expression. In addition, reporter systems enabling the tracing of cells carrying the modified gene of interest will be incorporated thereby refining the read out of the experiments.

Mouse strains carrying 'switch' gene alleles and reporter alleles will be referred to as 'tool box' strains. The functionality of these 'tool box' strains needs to be validated as well as functionality of the conditional modified mouse strains. Conditional genetic modifications should not lead to any phenotype but should serve efficiently as a substrate for 'switch' genes. Validation of these mouse strains and of alternative routes for 'switch' gene introduction will be described in this appendix 2.

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

For the validation of each ‘tool box’ strain, conditional strain or application to introduce ‘switch’ gene systems breeding pairs will be set up in order to obtain the experimental mice with the appropriate genotype. New ‘switch’ gene alleles will be combined with a validated reporter allele and new reporter alleles and new conditional alleles with a validated ‘switch’ gene allele. The validation experiments in case of somatic introduction of ‘switch’ gene systems will be directly done on validated reporter mice. Once the mice with the proper genotypes are available, the following procedures will be performed.

1) Tissue sampling of pups for genotyping and identification

a) toe clipping is performed at 5-7 days after birth OR

b) ear clipping is performed after weaning. This is done under anaesthesia according to SOP. The choice between toe- or ear-clip is dependent on the requirements or stage of breeding, as not always all mice need to be genotyped shortly after birth.

c) tail clipping after weaning under anaesthesia according to SOP (sometimes required to obtain sufficient DNA for careful assessment of the structure of transgene insertions).

2) Overall monitoring of mice for spontaneous phenotypic abnormalities, including regular weighing to monitor growth and possible development of obesity. Appearance, size, growth, behaviour, relative size, breeding parameters and clinical signs will all be assessed.

3) Generation of experimental cohorts in which the necessary genetic elements are combined (e.g. ‘switch’ gene alleles and reporter alleles).

4) Animals are euthanized according to SOP; tissue/cells will be subjected to further molecular and histological characterization directed at the following aspects:

-expression level and tissue/cell type specificity of ‘switch’ gene alleles

-functionality of ‘switch’ genes using validated reporter alleles as substrates

-inducibility of ‘switch’ genes using validated reporter alleles

-functionality of somatic delivery of switch genes using validated reporter alleles

-functionality of reporter alleles using validated ‘switch’ gene alleles

-functionality of conditional alleles

5) In case of inducible gene modification animals will be exposed to the appropriate inducing agents or control substances by one or more of the following routes (the best administration route and schedule is determined by the physicochemical nature/pharmacological properties of the inducing agent and the required extent and timing of the intended deletion):

a) in diet or drinking water, for maximally 2 weeks

b) intra-peritoneal injection (maximally 5 times)

c) oral administration (maximally 5 times)

d) topical application on the skin under anaesthesia according to SOP (maximally 3x)

6) For somatic introduction of gene modifications, various formulations of ‘switch’ gene systems can be used e.g. viral vector suspensions, liposome suspensions and DNA, RNA and proteins formulated in various solvents. In case the introduction of these systems will raise an immune response against the cells that have been targeted (e.g. viral vectors generating foreign antigens) we will supplement the drinking water of the mice with immune suppressants. For somatic introduction of the ‘switch’ gene systems, mice will be subjected to either one of the following procedures:

a) injection either sub-cutaneous, intra-peritoneal, intra-muscular, intra-venous, intra-thoracic; if necessary under anaesthesia according to SOP (1x)

b) introduction under anaesthesia according to SOP without surgery via either one of the openings in the

body, e.g. anal, urine tract, vaginal, intra-tracheal, inhalation, oral, milk duct mammary gland (1x)
c) surgical intra-cranial installation under anaesthesia and analgesia according to SOP (1x)
d) tattoo of the skin under anaesthesia according to SOP (maximally 3x).
e) shaving of the skin and topical application (ointment) (maximally 3x)
7) Blood sampling according to SOP at different time points after activation of the genetic modification.

None of the listed procedures causes a discomfort level more than 'mild' except for the surgical intra-cranial installation. Only a very small number of mice will be involved in this procedure (< 0,5%)

Welfare assessment:
Mice are monitored daily as part of the standard care by the Animal Facility. In addition the mice will be checked frequently by the researchers for the several parameters as described in the Directive 2010/63/EU: corrigendum of 24 January 2013. The parameters are: appearance, relative size, behaviour and posture, growth, coat condition, fertility and mortality figures, and clinical signs. In case of any discomfort more than mild the frequency will be increased.

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. All techniques are state-of-the-art and are proven effective in modulating inducible gene modification and characterizing GM mice with minimal numbers of animals.

B. The animals

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

Mus musculus: Wild-type and GM mice. Wild-type mice are in part obtained from registered commercial companies; all other mice are obtained from internal breeding programs. The institute is a license holder with NVWA.
We expect to functionally validate 25 new 'tool box' strains (10 carrying constitutive active 'switch' gene alleles, 10 inducible active 'switch' gene alleles and 5 new reporter alleles) and 25 new conditional alleles. In addition, based on the experiments during the last 5 years during which a broad range of conditional technologies has been applied, we fore see to analyse the effectiveness of 25 new strategies to somatically introduce 'switch' gene systems.
For functional analysis of new 'switch' alleles we have to set up groups of mice in which new 'switch' alleles are combined with validated 'reporter' alleles. This will be done by cross breeding which involves more mice due to Mendelian transmission (for each experimental mouse on average 4 mice are needed excluding parents)
To validate inducibility of 'switch' alleles we will test on average 5 conditions and the read out will be done using reporter mice as described above.
For validation of new 'reporter' alleles we have to set up groups of mice in which new 'reporter' alleles are combined with validated 'switch' alleles. This will be done by cross breeding which involves more mice due to Mendelian transmission (for each experimental mouse on average 4 mice are needed excluding parents).

For each new application to somatically introduce ‘switch’ alleles (e.g. viral) we will use on average 5 variables. For these experiments we will use validated ‘reporter’ strains. The nature of the variables depends on the application (e.g. dosage).
To validate the functionality of conditional alleles we will combine the new conditional allele with validated ‘switch alleles’. This will be done by cross breeding which involves more mice due to Mendelian transmission (for each experimental mouse on average 4 mice are needed excluding parents).

According to the *‘Herziene Wet op de Dierproeven: het genereren, fokken, genotyperen, monitoren en huisvesten van genetisch gewijzigde dieren’* (Versie: oktober 2016), a CCD license approval is needed only for experimental mice. Mice necessary for breeding do not have to be included.
The numbers of experimental and breeding mice involved in these experiments are listed below. The number of experimental mice are indicated in roman (and the number of additional breeding mice in italics).
The numbers are based on a group size of 5 animals for all of these analyses as in our experience this number is sufficient to draw firm conclusions.

nr mice needed	analysis		breeding	
Tool box lines				
Constitutive activity				
Expression analysis	5		*15*	
Functional analysis:	5		*30*	
Total per line	10		*45*	
3 lines per modification	30		*135*	
10 modifications		300		*1350*
Inducible activity				
Expression analysis	5		*15*	
Functional analysis (5 conditions)	25		*120*	
Total per line	30		*135*	
3 lines per modification	90		*405*	
10 modifications		900		*4050*
Reporters				
Expression analysis	5		*15*	
Functional analysis:	5		*30*	
Total per line	10		*45*	
3 lines per modification	30		*135*	
5 reporter lines		150		*625*
Somatic 'switch' gene introduction				
5 variables (e.g. dosage) + control	30			
25 applications		750		
Conditional alleles				
Recombination validation	5		*30*	
2 lines per cond. allele	10		*60*	
25 cond. alleles		250		*1500*
Total experimental:	2350			
Breeding				*7525*
Grand total:	9875			
Licensed in this application: 2350				

C. Re-use

Will the animals be re-used?

x No, continue with question D.

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

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

x 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.

- Generation and testing of a new 'tool box' and conditional lines is carefully considered in advance and should in principle yield new possibilities to study the effects of changes in gene activity in a complete animal.
- Before a new 'tool box' or conditional mouse line is created and tested we first extensively scan the literature and public resources (EMMA/Infrafrontier, JAX, KOMP and others) for already available 'tool box' strains and conditional strains of the same gene or locus. Duplicate mouse strains are not produced.

  In addition we will search the literature and other resources for the most efficient technologies for induction and somatic delivery.

  The publicly available resource (www.infrafrontier.eu) of genetically modified embryonic stem cells that our transgenics facility has created allows for the generation of compound GM mice without the need for subsequent breeding. This reduces the number of mice obtained by breeding efforts as all modified alleles are already present in the embryonic stem cells (Huijbers et al., Nat Protoc. 2015 Nov;10(11):1755-85. doi: 10.1038).
- The genetic modification will be performed as much as possible in the desired genetic background in which the subsequent experiments in this application (appendix 3 and 4) will be performed. This can be achieved by using gene editing technology in pre-implantation embryos and ES cells from the relevant background.

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

1) All mice are monitored daily for signs of discomfort.

2) There are no negative environmental effects; all mice are housed in controlled environments in individually ventilated cages (IVC). Mice get additional cage enrichment (for instance a mouse home and paper tissues) when housed solitarily (while minimized as much as possible, this is sometimes required during the genotype screening phase) and also when signs of distress are observed. In these cases monitoring will be intensified.

Repetition and duplication

E. Repetition

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

n.a.

Accommodation and care

F. Accommodation and care

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

x No

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

G. Location where the animals procedures are performed

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

x No > Continue with question H.

☐ Yes > Describe this establishment.

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

Classification of discomfort / humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

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

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

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

Anaesthesia according to SOP will be applied
-during ear or tail clipping,
-injection either intra-muscular, intra-thoracic.
-introduction via either one of the openings in the body, e.g. anal, urine tract, vaginal, intra-tracheal, inhalation, oral, milk duct mammary gland
-tattoo of the skin
Anaesthesia and analgesia will be applied according SOP
-during and after surgical intra-cranial installation

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 more adverse effects during cross-breeding of 'tool box' and conditional modified strains in the context of their validation.

Explain why these effects may emerge.

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

The mice are monitored daily. Action will be taken on the same day if unexpected adverse effects 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.

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

In case mice are assessed as having a spontaneous discomfort level higher than mild due to the genetic modification(s) they will not be continued and culled

Indicate the likely incidence.

Based on our experience so far the likelihood of this happening is low (only a very small fraction (<1%) of 'tool box' or conditional mouse strains).

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').

None of the mice that have undergone one of the listed procedures will suffer discomfort at a level more than 'mild' except for the surgical intra-cranial installation, which causes an estimated moderate discomfort. Only a very small minority of the mice will be involved in this procedure (< 0,5%).

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

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

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.

x Yes

Centrale Commissie Dierproeven

6

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.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'.	30100
1.2	Provide the name of the licenced establishment.	Stichting Het Nederlands Kanker Instituut – Antoni van Leeuwenhoek ziekenhuis

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
3	Phenotyping of mice carrying germ line or temporal and/or tissue specific genetic modifications

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.

To study the effects of gene modification in all cells of the mouse we will set up cohorts of germ line modified mice and appropriate controls. First we will monitor viability and Mendelian transmission. In case the germ line modification is viable we will follow the modified mice over time and monitor development and behaviour. In addition we will perform a thorough molecular and histo-pathological analysis at different ages. In cases of families of genes that have similar molecular activities (redundancy) or sets of complementing genes we might need to combine modification of multiple genes in order to uncover their physiological function. If germ line modification is not viable we will first characterize the phenotype at different stages of gestation. For this we will set up breeding pairs to produce pregnant females carrying modified and control foetuses.

In case we want to study gene function at later stages and in specific tissues/cell types we will take the conditional approach and combine the necessary genetic components to generate the experimental and control cohorts of mice by efficient breeding strategies. These genetic components include conditional alleles of the genes of interest and of mouse strains expressing various constitutive or inducible 'switch' genes (e.g. recombinases such as Cre, Flp) that activate the genetic modification in conditional genes.

Alternative approaches that introduce the 'switch' genes somatically will be involved as well. For example, the use of viral vectors carrying cell type specific promoters driving 'switch' gene expression. In addition, reporter systems enabling the tracing of cells carrying the modified gene of interest will be incorporated thereby refining the read out of the experiments.
Decision on timing and tissue/cell type specificity will be primarily based on the tumor type in which the gene of interest was found to be implicated in addition to their expression profile and other data. After inducing the gene modifications we will follow the mice over time and monitor development and behaviour. In addition we will perform a thorough molecular and histo-pathological analysis at on average 2 time points based on the expression and functional characteristics of the genetic modification of interest.

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

For the generation of the genetically modified and control cohorts breeding pairs will be set up in order to obtain the experimental mice with the appropriate genotype. For this the following procedures will be necessary:
1) Tissue sampling of pups for genotyping and identification
a) toe clipping performed at 5-7 days after birth OR
b) ear clipping performed after weaning. This is done under anesthesia according to SOP. The choice between toe- or ear-clip is dependent on the requirements or stage of breeding, as not always all mice need to be genotyped shortly after birth.
c) tail clipping after weaning under anesthesia according to SOP (sometimes required to obtain sufficient DNA for careful assessment of the structure of transgene insertions).
2) In case of inducible gene modification animals will be exposed to the appropriate inducing 'switch' gene activation agents or control substances by one or more of the following routes (the best administration route and schedule is determined by the physicochemical nature/pharmacological properties of the inducing agent and the required extent and timing of the intended deletion):
a) in diet or drinking water, for maximally 2 weeks
b) intra-peritoneal injection (maximally 5 times)
c) oral administration (maximally 5 times)
d) topical application on the skin under anesthesia according to SOP (maximally 3x).
3) For somatic introduction of gene modifications, various formulations of 'switch' gene systems can be used e.g. viral vector suspensions, liposome suspensions and DNA, RNA and proteins formulated in various solvents. In case the introduction of these systems will raise an immune response against the cells that have been targeted (e.g. viral vectors generating foreign antigens) we will supplement the drinking water of the mice with immune suppressants. For somatic introduction of the conditional gene, mice will be subjected to either one of the following procedures:
a) injection either sub-cutaneous, intra-peritoneal, intra-muscular, intra-venous, intra-thoracic; if necessary under anesthesia according to SOP (1x)
b) introduction under anesthesia according to without surgery via either one of the openings in the body, e.g. anal, urine tract, vaginal, intra-tracheal, inhalation, oral, milk duct mammary gland (1x)
c) surgical intra-cranial installation under anesthesia and analgesia according to SOP (1x)
d) tattoo of the skin under anesthesia according to (maximally 3x)
e) shaving of the skin and topical application (ointment) (maximally 3x)
Once the cohorts of mice carrying the relevant genetic modifications have been set up, either with or

without (somatic) induction, the following procedures will be performed:
4) Overall monitoring of mice for spontaneous phenotypic abnormalities, including regular weighing to monitor growth and possible development of obesity. Appearance, size, growth, behavior, relative size, breeding parameters and clinical signs will all be assessed.
5) Animals are killed according to SOP; tissue/cells will be harvested and for subjected to the following procedures:
-molecular characterization directed at the following aspects:

- reporter allele expression
- presence of gene modification of interest

-histo-pathology,
-tissue culture derivation
In some cases depending on the expression and functional characteristics of the genetic modification of interest, the following additional procedures will be performed:
6) Blood sampling according to SOP at different time points after activation of the genetic modification to measure a wide range of relevant blood parameters.
7) Mice are injected with DNA labeling substances (e.g. BrdU) before short before sacrifice in order to detect proliferating cells by histo-chemistry

None of the listed procedures causes a discomfort level more than 'mild' except for the surgical intra-cranial installation, which causes an estimated moderate discomfort. Only a very small minority of the mice will be involved in this procedure (< 0,5%).

Welfare assessment:
Mice are monitored daily as part of the standard care by the Animal Facility. In addition the mice will be checked frequently by the researchers for the several parameters as described in the Directive 2010/63/EU: corrigendum of 24 January 2013. The parameters are: appearance, relative size, behavior and posture, growth, coat condition, fertility and mortality figures, and clinical signs. In case of any discomfort more than mild the frequency will be increased.

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. All techniques are state-of-the-art and are proven effective in modulating inducible gene modification and characterizing GM mice with minimal numbers of animals.

B. The animals

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

Mus musculus: Wild-type and GM mice. Wild-type mice are in part obtained from registered commercial companies; all other mice are obtained from internal breeding programs. Our institute is a license holder with NVWA.
We expect to characterize 50 new lines carrying germ line modifications. Most of the modification will be homozygous. Controls for these experimental mice will be wild type littermates.
We expect to generate 5 compound modified mouse lines carrying modified redundant or complementing alleles. Generating the experimental mice will take significantly more mice in order to breed two modified alleles to homozygosity.
For 25 genes we expect to study their effects of conditional modification under, on average, 2 conditions

e.g. in a tissue specific and/or temporally controlled fashion. In these cases we will incorporate reporter alleles to trace the cells that have undergone the modification, which will need more mice for the generation of the experimental cohorts.
For 10 genes we expect to study their effects of conditional modification after somatic introduction of 'switch' genes. For these analyses we will also make use of reporter alleles to trace the cells that have undergone the modification. On average we expect to use 2 applications of 'switch' gene delivery.
Cohorts of experimental mice in which multiple modified alleles are combined will be generated by cross breeding. This will involve a considerable number of mice.
However, according to the *'Herziene Wet op de Dierproeven: het genereren, fokken, genotyperen, monitoren en huisvesten van genetisch gewijzigde dieren'* (Versie: oktober 2016), a CCD license approval is needed only for experimental mice. Mice necessary for breeding do not have to be included.
The numbers of experimental and breeding mice in this appendix are listed below. The number of experimental mice are indicated in roman (and the number of additional breeding mice in italics).
These numbers are based on a group size of 10 experimental and 10 control animals for all of these analyses as in our experience this number is sufficient to draw firm conclusions.

nr mice needed	experimental + controls		breeding	
Germline modification				
per line 1 time point	20		*24*	
3 time points	60		*72*	
2 lines each	120		*144*	
50 modified alleles		6000		*7200*
Compound germline modification				
per compound modified line	20		*96*	
3 time points	60		*288*	
10 compound modified lines		600		*2880*
Conditional modification				
per line + reporter	20		*48*	
2 time points	40		*96*	
2 'switch' alleles per line	80		*192*	
25 lines		2000		*4800*
Somatic 'switch' gene introduction				
per line + reporter	20		*48*	
2 applications	40		*96*	
10 lines		400		*960*
Total experimental	9000			
breeding				*15840*

Grand total: 24840
Licensed in this application: 9000

C. Re-use

Will the animals be re-used?

x No, continue with question D.

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

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

x 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.

- Generation and characterization of a new lines carrying modification in cancer related genes is carefully considered in advance and should in principle yield functional information that can not be obtained by 'in vitro' studies but only in a complete animal.
- Before a new mouse line is created and phenotyped we first extensively scan the literature and public resources (EMMA/Infrafrontier, JAX, KOMP and others) for already existing data on the function of the same gene or locus. We will not duplicate the generation of mouse strains.
  The publicly available resource (www.infrafrontier.eu) of genetically modified embryonic stem cells that our transgenics facility has created allows for the generation of compound GM mice without the need for subsequent breeding. This reduces the number of mice obtained by breeding efforts as all modified alleles are already present in the embryonic stem cells (Huijbers et al., Nat Protoc. 2015 Nov;10(11):1755-85. doi: 10.1038).
- The genetic modification will be performed as much as possible in the desired genetic background in which the experiments in this appendix will be performed. This can be achieved by using gene editing technology in pre-implantation embryos and ES cells from the relevant background.

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

1) All mice are monitored daily for signs of discomfort.
2) There are no negative environmental effects; all mice are housed in controlled environments in individually ventilated cages (IVC). Mice get additional cage enrichment (for instance a mouse home and paper tissues) when housed solitarily (while minimized as much as possible, this is sometimes required during the genotype screening phase) and also when signs of distress are observed. In these cases monitoring will be intensified.

Repetition and duplication

E. Repetition

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

n.a.

Accommodation and care

F. Accommodation and care

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

x No

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

G. Location where the animals procedures are performed

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

x No > Continue with question H.

☐ Yes > Describe this establishment.

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

Classification of discomfort / humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

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

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

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

Anaesthesia with isoflurane will be applied
-during ear or tail clipping,
-injection either intra-muscular, intra-thoracic.
-introduction surgery via either one of the openings in the body, e.g. anal, urine tract, vaginal, intra-tracheal, inhalation, oral, milk duct mammary gland
-tattoo of the skin
Anaesthesia and analgesia will be applied according SOP
-during and after surgical intra-cranial installation

I. Other aspects compromising the welfare of the animals

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

The purpose of this appendix is to study phenotypic consequences of (conditional) gene modification in living mice. Since the physiological function of the genes of interest is not known and in fact the aim of these studies and we cannot exclude the occurrence of adverse effects in some of the generated (compound) modified mouse strains. However, based on our 30 years' experience in this type of studies we expect that less than of 10% of the animals the phenotype will be affected and half of these might suffer unexpected discomfort. The nature of the adverse effects and level of discomfort is unpredictable. Rarely, mice are born (the F0 founders) with elephant teeth or showing a severely retarded development, but this also occurs during normal breeding.
In all cases mentioned above the affected animals will be killed immediately in order to limit the discomfort level to moderate

Explain why these effects may emerge.

In addition to the developmental and physiological consequences of the gene modifications for the handlings of the embryo's or ES cells (e.g. micro-injection or modification per se of ES cells) during the modification procedure might perhaps cause improper development in exceptional cases that emerge in the F0 founders.

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

The mice are monitored daily. Action will be taken on the same day if unexpected adverse effects 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.

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

In case mice are assessed as having a spontaneous discomfort level higher than moderate due to the genetic modification(s) they will not be continued and culled.

Indicate the likely incidence.

Based on our experience and published data (Dickinson et al. 2016, Nature 537: 508-514) only a small fraction (<5%) of genetically modified mouse strains might have spontaneous discomfort higher than mild.

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').

None of the mice that have undergone one of the listed procedures will suffer discomfort at a level more than 'mild' except for the surgical intra-cranial installation, which causes an estimated moderate discomfort. Only a very small number of the mice will be involved in this procedure (< 1%).

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

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

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.

x Yes

Centrale Commissie Dierproeven

7

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.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'.	30100
1.2	Provide the name of the licenced establishment.	Stichting Het Nederlands Kanker Instituut – Antoni van Leeuwenhoek ziekenhuis

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
		4	Functional analysis of genetic modifications in mice under challenging conditions

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.

For some genes the results of the analyses described in appendix 3 and existing data from other sources may indicate that their function can only be uncovered when the modified mice or tissues/cells derived from them are exposed to challenging conditions. This might be the case when they have a role is in stem cell performance supporting tissue homeostasis and regeneration. Such a role could be very relevant for tumor maintenance and post-treatment relapses. This appendix 4 describes short term (pilot) experiments involving challenging conditions to obtain the leads for separate, additional licence applications that address on a broad scale and in greater depth the function of these genes.
The conditions we want to apply enable to test in a defined and reproducible fashion the ability of genetically modified tissues/cells to contribute to the restoration of damaged tissues. This approach is especially suited to address the role of genes in (tissue) stem cell function. The tissues in which we intend to perform these analyses include the skin, mammary gland, the hematopoietic system and the liver. The research in our institute has a strong focus on tumorigenesis in these tissues, which are also most suitable for the analysis of stem cell performance, since these tissues have an intrinsically high regeneration potential.
We will perform these analyses only for genes for which we have indications that they are involved in

tissue homeostasis and regeneration and the regular approach described in appendix 3 is not informative.

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

Two types of animal experiments will be carried out:
1) Experiments in which genetically modified animals undergo tissue damaging procedures and in which restoration of these tissues will be analysed at different time points directly.
2) Experiments in which recipient animals undergo tissue damaging procedures and subsequently serve as acceptors for tissues/cells transplantations from genetically modified mice. The performance of these grafts in the recipient mice will be analysed, e.g. their ability to tissue restoration, at different time points.

For the type 1 experiments, we will perform the following tissue damaging procedures in experimental mice directly:
1) total body irradiation according to SOP
2) targeted, local irradiation to damage specific tissues according to SOP
3) local exposure to chemical agents (e.g. naphtaline damaging lung tissue); the method of application is determined by the physicochemical nature/pharmacological properties of the damaging agent
4) skin wounding under anaesthesia and analgesia according to SOP
5) partial mammary fat pad clearance under anaesthesia and analgesia according to SOP
6) partial hepatectomy under anaesthesia and analgesia according to SOP
In some cases using conditionally modified mice also carrying inducible 'switch' alleles the gene modification will be activated after the tissue damaging procedure. For this, animals will be exposed to the appropriate inducing 'switch' gene activation agents or control substances by one or more of the following routes (the best administration route and schedule is determined by the physicochemical nature/pharmacological properties of the inducing agent and the required extent and timing of the intended deletion):
a) in diet or drinking water, for maximally 2 weeks
b) intra-peritoneal injection (maximally 5 times)
c) oral administration (maximally 5 times)
d) topical application on the skin under isoflurane anesthesia (maximally 3x).

For the type 2 experiments, we will perform the following tissue damaging and transplantation procedures in recipient mice:
1) total body irradiation to deplete the hematopoietic system of the recipient mice followed by transplantation by intra-venous injection of bone marrow cell suspensions from genetically modified and control mice
2) surgical removal of small piece of the dorsal skin (wounding) of the recipient mice followed by transplantation skin tissue or skin cell suspensions from genetically modified and control mice under anaesthesia and analgesia in the wounded skin of recipient mice
3) mammary fat pad clearance of recipient mice and transfer of mammary gland tissue or mammary cell suspensions from genetically modified or control mice in cleared fat pads of recipient mice under anaesthesia and analgesia.
Transplanted tissues or cells may carry reporter alleles in order to accurately distinguish between

transplanted and host tissues/cells.
In some cases the gene modification in the donor tissue will may be activated after transplantation of tissues from conditionally modified mice carrying inducible 'switch' alleles. For this, after grafting the recipient animals will be exposed to the appropriate inducing 'switch' gene activation agents or control substances by one or more of the following routes (the best administration route and schedule is determined by the physicochemical nature/pharmacological properties of the inducing agent and the required extent and timing of the intended deletion):
a) in diet or drinking water, for maximally 2 weeks
b) intra-peritoneal injection (maximally 5 times)
c) oral administration (maximally 5 times)
d) topical application on the skin under isoflurane anesthesia (maximally 3x).

Once the cohorts of recipient mice carrying the relevant tissue damage have been set up, either with or without tissue/cell suspension transplantation, the following procedures will be performed:
-assessment of regeneration of the affected tissue by histopathology
-overall monitoring of mice for spontaneous phenotypic abnormalities, including regular weighing to monitor growth and possible development of obesity. Appearance, size, growth, behavior, relative size and clinical signs will all be assessed.
-i.p. injection with DNA labeling substances (e.g. BrdU) before sacrifice in order to detect proliferating cells
-animals are killed according to SOP; tissue/cells will be harvested and for subjected to the following procedures:

- molecular characterization directed at the following aspects:
    - reporter allele expression
    - presence of gene modification of interest
- histo-pathology,
- tissue culture derivation

Welfare assessment:
Mice are monitored daily as part of the standard care by the Animal Facility. In addition the mice will be checked frequently by the researchers for the several parameters as described in the Directive 2010/63/EU: corrigendum of 24 January 2013. The parameters are: appearance, relative size, behavior and posture, growth, coat condition, fertility and mortality figures, and clinical signs. In case of any discomfort more than mild the frequency will be increased.

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. All techniques are state-of-the-art and are proven effective in modulating inducible gene modification and characterizing GM mice with minimal numbers of animals.

B. The animals

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

Mus musculus: Wild-type and GM mice. Wild-type mice are in part obtained from registered commercial companies; all other mice are obtained from internal breeding programs. Our institute is a license holder with NVWA.

All mice will be enrolled as adult.
We expect to characterize the effects of 30 genetic modifications in type 1 experiments: 13 in mice after damaging by irradiation, 5 after tissue damaging by agents, 5 after skin wounding, 5 after partial mammary fat pad clearance and 2 after hepatectomy.
Per experiment we will use 10 experimental and 10 wild type control mice as in our experience these numbers are sufficient to draw significant conclusions.
In type 2 experiments we expect to analyze the transplants from 18 GM lines: 6 after bone marrow transplantation, 6 after skin/cell transplantation and 6 after mammary tissue/cell transplantation. For these transplantation experiments isogenic recipients will be used in order to immunologically match donor and recipient and to maximize the take rate of the transplants.
Per experiment we will use 10 recipients for transplantation of experimental tissue/cells and 10 recipients for transplantation of control tissue/cells as in our experience these numbers are sufficient to draw significant conclusions.

According to the *'Herziene Wet op de Dierproeven: het genereren, fokken, genotyperen, monitoren en huisvesten van genetisch gewijzigde dieren'* (Versie: oktober 2016), a CCD license approval is needed only for experimental mice. Mice necessary for breeding do not have to be included.
The numbers of experimental and breeding mice involved in these experiments are listed below. The number of experimental mice are indicated in roman (and the number of additional breeding mice in italics).

nr mice needed	experimental + controls		*breeding*	
Type1 experiment				
per GM line 1 time point	20		*72*	
3 time points	60		*216*	
30 GM lines		1800		*6480*
Type 2 experiment				
per GM tissue/cell transplant	20		*96*	
3 time points	60		*288*	
transplants from 18 GM lines		1080		*5184*
Total experimental		2880		
breeding				*11664*
Grand total:	14544			
Licensed in this application:	**2880**			

C. Re-use

Will the animals be re-used?

x No, continue with question D.

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

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

x 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 experiments to address the involvement of genes in stem cell performance and tissue regeneration using genetically modified mouse lines are carefully considered in advance and should in principle yield functional information that cannot be obtained by 'in vitro' studies but only in a complete animal. We will perform these experiments only for genes for which data from literature or our own results (e.g. from experiments described in appendix 3) strongly indicate a role in stem cell performance and tissue regeneration.
- All transplanted tissues or cells will carry reporter alleles in order to accurately distinguish between transplanted and host tissues/cells. This increases the sensitivity of the analyses and reduces the number of mice necessary for drawing sound conclusions
- In case of transplantation assays, if possible we will use recipient mice for both experimental and control transplant (e.g. skin transplantations on the left and right dorsal site). This improves the experimental setting and reduces the number of mice needed without increasing discomfort.
- The genetic modifications have been introduced in mice as much as possible in the desired genetic background in which the experiments in this appendix will be performed. This has been achieved by using gene editing technology in pre-implantation embryo's and ES cells from the relevant background.

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

1) All mice are monitored daily for signs of discomfort.
2) There are no negative environmental effects; all mice are housed in controlled environments in individually ventilated cages (IVC). Mice get additional cage enrichment (for instance a mouse home and paper tissues) when housed solitarily (while minimized as much as possible, this is sometimes required during the genotype screening phase) and also when signs of distress are observed. In these cases monitoring will be intensified.

Repetition and duplication

E. Repetition

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

n.a.

Accommodation and care

F. Accommodation and care

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

x No

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

G. Location where the animals procedures are performed

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

x No > Continue with question H.

☐ Yes > Describe this establishment.

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

Classification of discomfort / humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

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

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

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

Anaesthesia according to SOP will be applied when animals are exposed to

-total body and targeted, local irradiation

Anaesthesia and analgesia will be applied according SOP

-during and after surgical removal of small piece of the dorsal skin

-mammary fat pad clearance

-hepatectomy

Analgesia according to SOP will be applied

-after application of tissue damaging agents

I. Other aspects compromising the welfare of the animals

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

Depending on the damaging treatment mice might suffer from anaemia, local pain and general malaise.

Explain why these effects may emerge.

Irradiation causes bone marrow failure leading to anaemia and malaise; other tissue damaging treatments might cause pain due to local inflammatory lesions.

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

The mice are monitored daily. Action will be taken on the same day if unexpected adverse effects show up. In these cases mice will be taken out of the experiment when discomfort exceeds the level of moderate.

J. Humane endpoints

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

☐ No > Continue with question K.

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

We will use the criteria as described in the Code of Practice for cancer research.

Animals will be taken out of the experiment when discomfort exceeds the level of moderate

Indicate the likely incidence.

The likely incidence of exceeding discomfort level of moderate depends on the treatment. For this incidence we estimate the following for the treatments we will use:

total body irradiation:	<50%
exposure to damaging agents:	<5%
hepatectomy	<20%
other treatments	<5%

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').

For all experimental mice we expect a discomfort level of moderate. The mice are monitored daily. Animals will be taken out of the experiment when discomfort exceeds the level of moderate.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

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

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.

x Yes

Centrale Commissie Dierproeven

8

> Retouradres Postbus 20401 2500 EK Den Haag

Stichting Het Nederlands Kanker Instituut - Antoni van Leeuwenhoek ziekenhuis
[redacted]
Postbus 90203
1006 BE AMSTERDAM

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

Onze referentie
Aanvraagnummer
AVD301002017840

Bijlagen
2

Datum 23 januari 2017
Betreft Ontvangstbevestiging aanvraag projectvergunning Dierproeven

Geachte [redacted]

Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 20 januari 2017. Het gaat om uw project "Functional analysis of genes implicated in cancer". Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD301002017840. Gebruik dit nummer wanneer u contact met de CCD opneemt.

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

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

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

Datum:
23 januari 2017
Aanvraagnummer:
AVD301002017840

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlagen:
- Gegevens aanvraagformulier
- Factuur

Datum:
23 januari 2017
Aanvraagnummer:
AVD301002017840

Gegevens aanvrager

Uw gegevens	
Deelnemersnummer NVWA:	30100
Naam instelling of organisatie:	Stichting Het Nederlands Kanker Instituut - Antoni van Leeuwenhoek ziekenhuis
Naam portefeuillehouder of diens gemachtigde:	[redacted]
KvK-nummer:	40530817
Straat en huisnummer:	Plesmanlaan 121
Postbus:	90203
Postcode en plaats:	1006 BE AMSTERDAM
IBAN:	NL71DEUT0626343534
Tenaamstelling van het rekeningnummer:	Stichting Het Nederlands Kanker Instituut - Antoni van Leeuwenhoek Ziekenhuis

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

Datum:
23 januari 2017
Aanvraagnummer:
AVD301002017840

Gegevens verantwoordelijke uitvoering proces

Naam:	[redacted]
Functie:	Instantie voor Dierenwelzijn
Afdeling:	[redacted]
Telefoonnummer:	[redacted]
E-mailadres:	[redacted]

Over uw aanvraag

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

Over uw project

Geplande startdatum:	1 februari 2017
Geplande einddatum:	1 februari 2022
Titel project:	Functional analysis of genes implicated in cancer
Titel niet-technische samenvatting:	Functionale analyse van genen betrokken bij kanker
Naam DEC:	NKI
Postadres DEC:	t.a.v. [redacted] Postbus 90203;1006 BE; Amsterdam
E-mailadres DEC:	[redacted]

Betaalgegevens

De leges bedragen:	€ 1.684,-
De leges voldoet u:	na ontvangst van de factuur

Checklist bijlagen

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

Ondertekening

Naam:	[redacted]
Functie:	Instantie voor dierenwelzijn
Plaats:	Amsterdam
Datum:	20 januari 2017

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Nederlands Kanker Instituut
t.a.v. [redacted]
Postbus 90203
1006 BE AMSTERDAM

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

Onze referentie
Aanvraagnummer
AVD301002017840

Bijlagen
2

Datum 23 januari 2017
Betreft Factuur aanvraag projectvergunning Dierproeven

Factuur
Factuurdatum: 23 januari 2017
Vervaldatum: 22 februari 2017
Factuurnummer: 170840
Ordernummer: Cost center 4050 / Project 16 006

Omschrijving	**Bedrag**
Betaling leges projectvergunning dierproeven Betreft aanvraag AVD301002017840	€ 1.684,00

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

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Stichting Het Nederlands Kanker Instituut - Antoni van Leeuwenhoek ziekenhuis
[redacted]
Postbus 90203
1006 BE AMSTERDAM

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

Onze referentie
Aanvraagnummer
AVD301002017840

Datum
Betreft Vervolg aanvraag projectvergunning Dierproeven

Geachte [redacted]

Op 20 januari 2017 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Functional analysis of genes implicated in cancer" met aanvraagnummer AVD301002017840. Uw aanvraag wordt in behandeling genomen. In deze brief leest u wanneer u een beslissing kunt verwachten.

Wanneer een beslissing
Wij nemen uiterlijk 17 maart 2017 een beslissing. Omdat een DEC-advies is meegestuurd met de aanvraag, streven wij ernaar om de aanvraag binnen 20 werkdagen te beslissen. Als wij nog informatie nodig hebben, kan dit later worden. Voor een complexe aanvraag staat een langere termijn. In beide gevallen ontvangt u daarover bericht. Als u goedkeuring krijgt op uw aanvraag, kunt u daarna beginnen met het project.

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

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

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Van: Info-zbo
Verzonden: dinsdag 7 februari 2017 15:02
Aan: [redacted]
CC: [redacted]
Onderwerp: Aanvraag AVD301002017840
Bijlagen: AanhoudenBeoordelenBrief_1.pdf

Geachte [redacted]
Bijgevoegde brief wordt u niet meer per post toegezonden.
In principe heeft u 14 dagen de tijd om op de vragen te antwoorden. Indien wij uiterlijk 15 februari de antwoorden binnen hebben, zal deze aanvraag in de eerstvolgende CCD vergadering besproken worden.

Met vriendelijke groet,

[redacted]

Centrale Commissie Dierproeven www.centralecommissiedierproeven.nl

Postbus 20401 | 2500 EK | Den Haag

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

Centrale Commissie Dierproeven

11

> Retouradres Postbus 20401 2500 EK Den Haag

Stichting Het Nederlands Kanker Instituut - Antoni van Leeuwenhoek ziekenhuis
[redacted]
Postbus 90203
1006 BE AMSTERDAM

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

Onze referentie
Aanvraagnummer
AVD301002017840

Datum 7 februari 2017
Betreft Aanvulling aanvraag projectvergunning Dierproeven

Geachte [redacted]

Op 20 januari 2017 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Functional analysis of genes implicated in cancer" met aanvraagnummer AVD301002017840. In uw aanvraag zitten voor ons nog enkele onduidelijkheden. In deze brief leest u wat wij nog nodig hebben en wanneer u een beslissing kunt verwachten.

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

Niet technische samenvatting
In de NTS heeft u niets gezegd over het feit dat voor deze experimenten nieuwe muizen stammen worden gemaakt, waarbij ook een deel van de dieren niet gebruikt zal worden. Om een volledig beeld te geven van wat gebeurt in dit project verzoeken wij u dit toe te voegen.

Onduidelijkheden
1) Bij de criteria van humane eindpunten die u gebruikt schrijft u "when discomfort exceeds the level of moderate". Kunt u nader omschrijven hoe dit gedefiniëerd wordt.

2) Bij de verschillende bijlagen heeft u vraag L niet ingevuld. Graag dit alsnog doen.

3) Kunt u aangeven voor bijlagen 3.4.4.2, 3.4.4.3 en 3.4.4.4 of u dieren van beide geslachten zult gebruiken. Indien u enkel 1 geslacht gebruikt in deze bijlagen, kunt u dit dan onderbouwen?

Zonder deze aanvullende informatie kan de beslissing nadelig voor u uitvallen omdat de gegevens onvolledig of onduidelijk zijn.

Opsturen binnen veertien dagen
Stuur de ontbrekende informatie binnen veertien dagen na de datum van deze brief op. U kunt dit aanleveren via NetFTP. Stuurt u het per post op, gebruik dan het formulier dat u bij deze brief krijgt.

Wanneer een beslissing
De behandeling van uw aanvraag wordt opgeschort tot het moment dat wij de aanvullende informatie hebben ontvangen. Als u goedkeuring krijgt op uw aanvraag, kunt u daarna beginnen met het project.

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

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlagen:
- Melding bijlagen
- Niet technische samenvatting

Centrale Commissie Dierproeven

Melding bijlagen

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.centralecommissiedierproeven.nl
Of bel met ons: 0900 28 000 28 (10 ct/min).

1 Uw Gegevens

Naam instelling:	Stichting Het Nederlands Kanker Instituut - Antoni van Leeuwenhoek ziekenhuis
Adres:	.....
Postcode en plaats:	.....
Aanvraagnummer:	AVD301002017840

2 Bijlagen
Welke bijlagen stuurt u mee?
Vink de bijlagen aan of vul de naam of omschrijving in.

O Projectvoorstel

O Beschrijving Dierproeven

O Niet-technische samenvatting

O Melding Machtiging

O Aanvraagformulier

O .....

O .....

O .....

Datum:
7 februari 2017
Aanvraagnummer:
AVD301002017840

3 Ondertekening

Naam: .....

Datum: ..... - ..... - .....

Handtekening: .....

Onderteken het formulier en stuur het met alle bijlagen op naar:
Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag

Van: Info-zbo
Verzonden: dinsdag 7 februari 2017 15:09
Aan: [redacted]
Onderwerp: Aanvraag AVD301002017840
Bijlagen: AanhoudenBeoordelenBrief_1.pdf

Geachte DEC,
Enige tijd geleden ontvingen wij een aanvraag waarover uw DEC een advies heeft uitgebracht. Het gaat om een aanvraag getiteld: Functional analysis of genes implicated in cancer met aanvraagnummer AVD301002017840. Wij hebben de aanvrager nog enkele vragen gesteld (zie bijgevoegde brief).

Indien u als DEC nog aanvullend wilt adviseren op deze vragen, kan dat.

Daarnaast willen wij u vragen op basis waarvan u adviseert om een beoordeling achteraf toe te voegen aan deze aanvraag. Doelt u hiermee op rapportage over de fokaantallen? Graag verheldering.

Wij willen u verzoeken om uiterlijk 15 februari te reageren op deze e-mail, zodat deze aanvraag in de eerstvolgende CCD vergadering kan worden besproken.

Met vriendelijke groet,

[redacted]

Centrale Commissie Dierproeven www.centralecommissiedierproeven.nl

Postbus 20401 | 2500 EK | Den Haag

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

13

Van: Info-zbo
Verzonden: vrijdag 10 februari 2017 15:46
Aan: [redacted]
CC: [redacted]
Onderwerp: RE: Aanvraag AVD301002017840

Geachte [redacted]
Zoals vanochtend telefonisch besproken met [redacted], kunt u van de brief die ik op 7 februari per e-mail verstuurd heb, de vraag over de NTS negeren.
Deze vraag is niet van toepassing op uw aanvraag, voor zover ik dit nu kan beoordelen.

Met vriendelijke groet,

[redacted]

Centrale Commissie Dierproeven www.centralecommissiedierproeven.nl

Postbus 20401 | 2500 EK | Den Haag
T: 0900 2800028
E: info@zbo-ccd.nl

Van: Info-zbo
Verzonden: dinsdag 7 februari 2017 15:02
Aan: [redacted]
CC: [redacted]
Onderwerp: Aanvraag AVD301002017840

Geachte [redacted]
Bijgevoegde brief wordt u niet meer per post toegezonden.
In principe heeft u 14 dagen de tijd om op de vragen te antwoorden. Indien wij uiterlijk 15 februari de antwoorden binnen hebben, zal deze aanvraag in de eerstvolgende CCD vergadering besproken worden.

Met vriendelijke groet,

[redacted]

Centrale Commissie Dierproeven www.centralecommissiedierproeven.nl

Postbus 20401 | 2500 EK | Den Haag
T: 0900 2800028
E: info@zbo-ccd.nl

14

Van: [redacted]
Verzonden: dinsdag 14 februari 2017 18:07
Aan: info@zbo-ccd.nl
Onderwerp: RE: Aanvraag AVD301002017840

Opvolgingsmarkering: Opvolgen
Markeringsstatus: Voltooid

Categorieën: Dossier: [redacted]

Geachte [redacted]

Graag attendeer ik u erop dat de DEC NKI haar reactie vanmiddag heeft geuploaded in het CCD-systeem

Mocht u nog vragen hebben, hoor ik dit graag.

Bij voorbaat veel dank.

Met vriendelijke groet,

[redacted]

The Netherlands Cancer Institute | Plesmanlaan 121 | 1066 CX AMSTERDAM | www.nki.nl
Antoni van Leeuwenhoek | Plesmanlaan 121 | 1066 CX AMSTERDAM | www.avl.nl

Dit e-mailbericht is uitsluitend bestemd voor de geadresseerde(n). Als dit bericht niet voor u bestemd is, wordt u vriendelijk verzocht dit aan de afzender te melden. Het Antoni van Leeuwenhoek (AVL) staat door de elektronische verzending van dit bericht niet in voor de juiste en volledige overbrenging van de inhoud, noch voor tijdige ontvangst daarvan. Voor informatie over het AVL raadpleegt u www.avl.nl

---

From: Info-zbo [mailto:info@zbo-ccd.nl]
Sent: dinsdag 7 februari 2017 15:09
To: DEC
Subject: Aanvraag AVD301002017840

Geachte DEC,
Enige tijd geleden ontvingen wij een aanvraag waarover uw DEC een advies heeft uitgebracht. Het gaat om een aanvraag getiteld: Functional analysis of genes implicated in cancer met aanvraagnummer AVD301002017840. Wij hebben de aanvrager nog enkele vragen gesteld (zie bijgevoegde brief).

Indien u als DEC nog aanvullend wilt adviseren op deze vragen, kan dat.

Daarnaast willen wij u vragen op basis waarvan u adviseert om een beoordeling achteraf toe te voegen aan deze aanvraag. Doelt u hiermee op rapportage over de fokaantallen? Graag verheldering.

Wij willen u verzoeken om uiterlijk 15 februari te reageren op deze e-mail, zodat deze aanvraag in de eerstvolgende CCD vergadering kan worden besproken.

Met vriendelijke groet,

[redacted]

Centrale Commissie Dierproeven **www.centralecommissiedierproeven.nl**

.....
Postbus 20401 | 2500 EK | Den Haag
.....
T: 0900 2800028
E: info@zbo-ccd.nl

Dierexperimentencommissie NKI
Plesmanlaan 121
1066 CX AMSTERDAM
[redacted]

NETHERLANDS CANCER INSTITUTE
ANTONI VAN LEEUWENHOEK

DEC advies aan CCD

A. Algemene gegevens over de procedure

1. Aanvraagnummer:
2. Titel van het project: Functional analysis of genes implicated in cancer
3. Titel van de NTS: Functionele analyse van genen betrokken bij kanker

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

5. Contactgegevens DEC:
   - naam DEC: DEC NKI
   - telefoonnummer contactpersoon: [redacted] bereikbaar op [redacted]
   - e-mailadres contactpersoon: [redacted]

6. Adviestraject (data dd-mm-jjjj):
   ☒ ontvangen door DEC: 07-12-2016
   ☐ aanvraag compleet
   ☒ in vergadering besproken: 14-12-2016
   ☐ anderszins behandeld
   ☒ termijnonderbreking(en) van 14-12-2016 t/m 11-01-2017
   ☐ besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen
   ☒ aanpassing aanvraag: 11-01-2017
   ☒ advies aan CCD: 20-01-2017

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

8. Eventueel horen van aanvrager:
   - Datum: 14-12-2016
   - Plaats: NKI
   - Aantal aanwezige DEC-leden: 5
   - Aanwezige (namens) aanvrager: [redacted]

9. Correspondentie met de aanvrager
   - Datum: 14 december 2016
   - Gestelde vragen en antwoorden:
   - De aanvrager is verzocht om in het projectvoorstel bij vraag 3.2 duidelijk aan te geven wat de interne samenhang van dit project is. *De tekst is aangepast.*
   - De aanvrager is verzocht om de passage over het maatschappelijk belang bij vraag 3.3 van het projectvoorstel te herschrijven. *De tekst is aangepast.*
   - De aanvrager is verzocht om in de handreiking van begin oktober 2016 zorgvuldig na te gaan of het nodig is om de dieren betrokken bij de verplichte welzijnsmonitoring van de

gegenereerde lijnen onderdeel te maken van dit project. *De aanvrager heeft de aanvraag op dit punt ongewijzigd gelaten. De DEC kan zich vinden in de gehanteerde interpretatie van de handreiking.*
- De aanvrager heeft tal van redactionele suggesties ontvangen. *De suggesties zijn waar nodig adequaat verwerkt in de aanvraag.*

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

B. Beoordeling (adviesvraag en behandeling)

1. Het project is vergunningplichtig (dierproeven in de zin der wet).
2. De aanvraag betreft een nieuwe aanvraag.
3. De DEC is competent om hierover te adviseren.
4. Eén van de DEC-leden is betrokken bij deze projectaanvraag. Dit lid is uitgesloten van de besluitvorming over deze aanvraag.

C. Beoordeling (inhoud)

1. Deze aanvraag heeft een concrete doelstelling en kan getypeerd worden als een project. De opzet komt het best overeen met voorbeeld 4b uit de handreiking 'Invulling definitie project' van de CCD. Het is helder welke handelingen individuele dieren zullen ondergaan. Hierdoor is ook duidelijk welk ongerief individuele dieren zullen ondergaan.
   Het kankeronderzoek, zowel in tumormateriaal van patiënten als in proefdieren, resulteert in de identificatie van grote aantallen genen en genetische elementen die betrokken zijn bij tal van vormen van kanker. De stap die daarop volgt is fundamenteel onderzoek naar de functie van die genen in normale fysiologische omstandigheden. Dit project heeft betrekking op die stap. Er worden genetisch gemodificeerde muizenlijnen gegenereerd (onder een andere vergunning) of geïmporteerd, waarin de door de aanvrager geselecteerde genen veranderd tot expressie komen. Deze muizenlijnen worden in het kader van dit project gefenotypeerd en op basis van de resultaten wordt een besluit genomen over de vraag of verder onderzoek naar de rol van de betreffende genen bij ontstaan en verloop van kanker en naar gerichte therapeutische interventies gerechtvaardigd is. Dit project omvat tevens de welzijnsbewaking van nieuw tot stand gekomen genetisch gemodificeerde lijnen voor zover verwacht wordt dat daarbij sprake zal zijn van een aangetast fenotype. Op basis van ervaring weet de aanvrager dat dit bij maximaal 10% van de lijnen het geval zal zijn.
   Dit onderzoek levert voor alle onderzochte genen de informatie op die nodig is om een "go/no go" beslissing te kunnen nemen over verder onderzoek en om beslissingen te kunnen nemen over het design van dat vervolgonderzoek (waarvoor dan een aparte vergunningaanvraag zal worden ingediend).
   Aangezien verwacht mag worden dat in relatief veel gevallen dit onderzoek tot een "no go" leidt, of tot een aanzienlijke aanpassing van het vervolgonderzoek, is het naar de mening van de DEC gerechtvaardigd om al dit eenvoudige en routinematige preliminaire onderzoek naar de normale fysiologische rol van genen in één aanvraag op te nemen (horizontale benadering). Het indienen van een groot aantal verschillende projectaanvragen voor losse (groepen van) genen, waarin dan ook het vervolgonderzoek naar die genen is opgenomen (verticale benadering), zou er toe leiden

dat in veel projecten al in een vroeg stadium een “no go” beslissing genomen wordt. Ook zou het vervolgonderzoek in deze verticale projectaanvragen noodzakelijkerwijs nog in algemene termen beschreven moeten worden. Met de horizontale benadering wordt voorkomen dat er een groot aantal aanvragen ingediend wordt met in algemene termen beschreven vervolgonderzoek dat mogelijkerwijs uiteindelijk niet zal worden uitgevoerd.
De DEC is er van overtuigd dat de aanvrager gedurende het project op zorgvuldige wijze besluiten zal nemen over de voortgang van het project en dat er niet onnodig dieren gebruikt zullen worden.

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

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

Belangen en waarden

4. Het directe doel van het project is het onderzoeken van de normale functie van genen waarvan gebleken is dat die ontregeld zijn bij kanker. Hiertoe worden modificaties in deze genen geïntroduceerd in muizen, of worden muizen met genetische modificaties geïmporteerd en gefokt, en worden vervolgens de gevolgen van die modificaties voor de normale ontwikkeling van deze muizen geanalyseerd. Deze kennis van de normale functie van de genen is onmisbaar om vast te kunnen stellen wat er fout gaat als die genen, door het feit dat ze ontregeld zijn, bijdragen aan het ontstaan van kanker. Het uiteindelijke doel is om langs deze weg aanknopingspunten te vinden voor therapeutische interventies. Het verband tussen het directe doel en het uiteindelijke doel is weliswaar niet direct, het betreft immers fundamenteel onderzoek, maar wel reëel. Het doel van deze projectaanvraag is gerechtvaardigd binnen de context van het onderzoeksveld, omdat er binnen dat veld consensus is over de waarde van deze aanpak.

5. De belangrijkste belanghebbenden in deze projectaanvraag zijn de proefdieren, de onderzoekers en de doelgroep/patiënten.
Voor de proefdieren geldt dat hun welzijn en integriteit worden aangetast. De dieren zullen beperkt worden in hun natuurlijke gedrag en gedurende de proeven zullen de dieren stress ondervinden en pijn ondergaan. Uiteindelijk zullen ze in het kader van het onderzoek gedood worden. De dieren hebben er belang bij hiervan gevrijwaard te blijven.
Voor de onderzoekers geldt dat ze belangrijke nieuwe wetenschappelijke inzichten kunnen verkrijgen en publiceren, hetgeen vaak de sleutel is tot het verkrijgen van nieuwe onderzoeksmiddelen en -mogelijkheden. Naar de mening van de DEC dient dat echter geen rol te spelen in de ethische afweging over de toelaatbaarheid van het gebruik van proefdieren. Het gaat uiteindelijk om de vraag of dit onderzoek belangrijke maatschappelijke en wetenschappelijke doelen dient (gezondheid, kennis). Dit onderzoek is in de eerste plaats fundamenteel van aard en levert informatie en kennis op die van belang is voor de voortgang van het onderzoek in dit veld.
Voor kankerpatiënten is dit onderzoek van belang, omdat het op termijn kan bijdragen aan een verbetering van de mogelijkheden om kanker te behandelen. Mechanistisch inzicht kan bijdragen aan een gerichte behandeling en diagnostiek met minder bijwerkingen. Dit kan er toe leiden dat de patiënt weer gezond wordt, dan wel een betere kwaliteit van leven heeft. Kunnen beschikken over een breed palet van mogelijke therapieën voor kanker, een ernstige aandoening die zich in een groot aantal vormen manifesteert, is ook van groot belang voor de samenleving.

6. Er is geen sprake van belangwekkende milieueffecten.

Proefopzet en haalbaarheid

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

8. De doelstellingen van het project zijn realistisch en de voorgestelde experimentele opzet en uitkomstparameters sluiten hier logisch bij aan. De DEC is dan ook van mening dat het project goed is opgezet, en dat deze strategie en experimentele aanpak kunnen leiden tot het behalen van de doelstellingen binnen het kader van het project.

Welzijn dieren

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

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

11. Het cumulatieve ongerief als gevolg van de dierproeven is realistisch ingeschat en geclassificeerd. Het ongerief wordt bij het grootste deel van de dieren (73%) bepaald door de weinig ingrijpende handelingen die nodig zijn om de dieren te genotyperen en te fenotyperen. In ca. 10% van de genetische gemodificeerde lijnen die worden onderzocht zal naar verwachting sprake zijn van licht tot maximaal matig ongerief als gevolg van die modificatie. Waar dat te voorzien is wordt, indien mogelijk, gebruik gemaakt van conditionele genetische modificaties. In gevallen waarin het onderzoek aanwijzingen oplevert dat bepaalde genen betrokken kunnen zijn bij weefselhomeostase kan het voor de initiële fenotypering noodzakelijk zijn om weefsels te beschadigen om zo te achterhalen of de betreffende genen een rol spelen bij het herstel. De DEC schat de ernst hiervan in als matig, mede op grond van het feit dat strikte humane eindpunten zullen worden gehanteerd die moeten voorkomen dat het ongerief zich verder ontwikkelt tot ernstig.
    Het cumulatief ongerief voor de dieren is dus juist ingeschat als licht voor 73% van de dieren, matig voor 27% van de dieren.

12. Elke dierproef brengt instrumenteel gebruik van speciaal voor dat doel in gevangenschap gefokte dieren met zich mee, hetgeen op zich al opgevat kan worden als een aantasting van hun integriteit. Omdat dit voor elk project geldt, vermeldt de DEC hier alleen zaken die kenmerkend zijn voor dit specifieke project. De integriteit van de dieren wordt of is aangetast op het niveau van het genoom door het aanbrengen van genetische modificaties, waarna men op zoek gaat naar (vaak subtiele) fenotypische veranderingen die een aanwijzing geven over de normale functie van het gen waarvan de expressie gewijzigd is. Behoudens uitzonderingen, valt niet te

verwachten dat dit tot ingrijpende fenotypische veranderingen leidt. De commissie is daarom van mening dat er sprake is van een beperkte aantasting van de integriteit.

13. Normaal gesproken valt in het grootste deel van dit onderzoek niet te verwachten dat dieren een humaan eindpunt zullen bereiken, omdat de genetische modificatie ernstige gevolgen blijkt te hebben. Het percentage dieren dat naar verwachting een humaan eindpunt zal bereiken is lager dan 1% en is op basis van eerdere ervaringen ingeschat. In veel van die gevallen is de reden om het dier uit de proef te nemen niet gerelateerd aan het experiment of de genetische modificatie, maar algemeen van aard. In bijlage 4 zal naar verwachting wel een aanzienlijk deel van de dieren uit de proef genomen worden, omdat verwacht mag worden dat het ongerief anders hoger dan matig zal worden. De risico's en symptomen die gepaard gaan met de verschillende "challenges" in deze bijlage zijn echter in grote lijnen bekend, zodat adequate monitoring en tijdig ingrijpen mogelijk is. De criteria voor humane eindpunten zijn voldoende specifiek gedefinieerd en toegesneden op de experimenten. De commissie is het eens met de inschattingen en met de gehanteerde humane eindpunten.

3V's

14. De aanvrager heeft voldoende aannemelijk gemaakt dat er geen geschikte vervangingsalternatieven zijn. Meestal zijn de aanwijzingen dat bepaalde genen bij kanker betrokken zouden kunnen zijn afkomstig uit (ex vivo) onderzoek aan tumorweefsel van patiënten en proefdieren. Waar mogelijk wordt de functie van die genen (eerst) in vitro onderzocht. Uiteindelijk vergt echter het onderzoeken van de rol van een gen in de normale ontwikkeling een intact dier waarin de expressie van dat gen is veranderd. Het is niet mogelijk om de vraagstellingen van dit project volledig zonder proefdieren te beantwoorden.

15. Het maximale aantal te gebruiken dieren is realistisch ingeschat en is proportioneel ten opzichte van de gekozen onderzoeksopzet en de looptijd. De onderzoekers hanteren een goede strategie om ervoor te zorgen dat er met het kleinst mogelijk aantal dieren wordt gewerkt waarmee nog een wetenschappelijk betrouwbaar resultaat kan worden verkregen. Door de stapsgewijze aanpak wordt onnodig gebruik van proefdieren voorkomen. De aanvrager heeft inzichtelijk gemaakt hoe en in welke mate dit onderzoek leidt tot fokoverschotten. Het betreft dieren die wel geboren worden in het kader van dit onderzoek, maar niet gebruikt worden in de experimenten (bijvoorbeeld omdat zij niet het juiste genotype hebben). Naar het oordeel van de DEC zijn deze fokoverschotten niet te vermijden.

16. Het project is in overeenstemming met de vereiste van de verfijning van dierproeven. De dieren worden zo kort mogelijk in het experiment gehouden en er worden adequate humane eindpunten gehanteerd. Het gebruik van genetisch gemodificeerde dieren waarbij de genetische modificaties weefselspecifiek en tamoxifen-induceerbaar zijn voorkomt onnodig ongerief door ongecontroleerde tumorgroei. De DEC is ervan overtuigd dat de beschreven proefopzet de meest verfijnde is en dat de dierproeven zo humaan mogelijk worden uitgevoerd.

17. Het project betreft geen wettelijk vereist onderzoek.

Dieren in voorraad gedood en bestemming dieren na afloop proef

18. De aanvrager zal in het project in de regel gebruik maken van zowel mannelijke, als vrouwelijke dieren, tenzij het onderzoek betreft naar genen die een rol spelen bij tumoren die geslachtsgebonden zijn.

19. De dieren zullen in het kader van het project gedood worden. Dit is noodzakelijk om verschillende weefsels en organen na afloop te kunnen uitnemen voor verder onderzoek. De gebruikte dodingsmethode staat vermeld in bijlage IV van richtlijn 2010/63/EU.

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

    NTS

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

D. Ethische afweging

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

2. Voor alle dieren vindt een lichte of matige aantasting van welzijn en integriteit plaats (beschreven in C9 tot C20). De doelstellingen kunnen niet zonder dieren behaald worden. De onderzoekers doen al het mogelijke om het lijden van de dieren en het aantal dieren te beperken.
   Doel van het project is het onderzoeken van de normale functie van genen waarvan gebleken is dat die ontregeld zijn bij kanker. Deze kennis van de normale functie van de genen is onmisbaar om vast te kunnen stellen wat er fout gaat als die genen, door het feit dat ze ontregeld zijn, bijdragen aan het ontstaan van kanker. Het uiteindelijke doel is om langs deze weg aanknopingspunten te vinden voor therapeutische interventies. Er is dringend behoefte aan nieuwe therapeutische benaderingen voor kanker die in combinatie met andere therapieën kunnen worden ingezet om zo de kansen op genezing, of het onder controle houden van de ziekte, te vergroten. Op termijn is het onderzoek daarmee ook voor patiënten en voor de samenleving van belang, omdat het kan bijdragen aan een verbetering van de gezondheid en kwaliteit van leven van veel mensen. De DEC acht het doel van het onderzoek om deze redenen van groot belang.

3. De DEC is overtuigd van het grote belang van de doelstelling van dit project. De commissie is daarnaast overtuigd van de kwaliteit van het onderzoek van de aanvrager. Dit onderzoek is ingebed in een gerenommeerd instituut dat over alle noodzakelijke voorzieningen beschikt. De DEC is van mening dat het project goed is opgezet, en dat de gekozen strategie en experimentele aanpak kunnen leiden tot het behalen van de doelstelling binnen het kader van het project. De aanvrager heeft voldoende aannemelijk gemaakt dat er geen geschikte vervangingsalternatieven zijn, dat het doel niet met minder dieren behaald kan worden, dat de gebruikte aanpak de meest verfijnde is en dat zij zal kunnen voorkomen dat mens, dier en het milieu onbedoelde negatieve effecten ondervinden als gevolg van de dierproeven.
   De DEC is van oordeel dat het hierboven geschetste grote belang van de doelstelling de onvermijdelijke nadelige gevolgen van dit onderzoek voor de dieren, in de vorm van angst, pijn of stress, rechtvaardigt. Aan de eis dat het belang van het onderzoek op dient te wegen tegen het ongerief dat de dieren wordt berokkend, is voldaan.

E. Advies

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

   ☒ De DEC adviseert de vergunning te verlenen onder de volgende voorwaarden
      - Op grond van het wettelijk vereiste (art. 10a1, lid 3) dient de projectleider bij beëindiging van het project een beoordeling achteraf aan te leveren die is afgestemd met de IvD.

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

2. Het uitgebrachte advies is gebaseerd op consensus.

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

Met vriendelijke groet,

[redacted]

ambt. secretaris DEC/NKI

[redacted]

16

Dierexperimentencommissie NKI
Plesmanlaan 121
1066 CX AMSTERDAM
[redacted]

NETHERLANDS CANCER INSTITUTE
ANTONI VAN LEEUWENHOEK

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14 februari 2017

Betreft: Aanvraag AVD301002017840

Geachte CCD,

Het advies om deze vergunning te verlenen onder de voorwaarde dat er achteraf een beoordeling plaats dient te vinden, is afkomstig uit een concept-versie van het advies en ten onrechte in de definitieve versie blijven staan. In het eerste concept van het advies werd er van uit gegaan dat er bij een klein deel van de dieren in bijlage 4 kortdurend ernstig ongerief op zou kunnen treden. Tijdens de bespreking van de aanvraag door de voltallige DEC bleek dat de DEC-leden vonden dat bij een strikte toepassing van humane eindpunten geen ernstig ongerief zou hoeven optreden. De commissie heeft dit ook expliciet verwoord in haar advies (citaat C13): *'In bijlage 4 zal naar verwachting wel een aanzienlijk deel van de dieren uit de proef genomen worden, omdat verwacht mag worden dat het ongerief anders hoger dan matig zal worden. De risico's en symptomen die gepaard gaan met de verschillende "challenges" in deze bijlage zijn echter in grote lijnen bekend, zodat adequate monitoring en tijdig ingrijpen mogelijk is. De criteria voor humane eindpunten zijn voldoende specifiek gedefinieerd en toegesneden op de experimenten'.*

De DEC doet u hierbij een herziene versie van het advies toekomen waaruit het advies om een voorwaarde op te nemen verwijderd is.

Met vriendelijke groet,

[redacted]

ambt. secretaris DEC/NKI
[redacted]l

Dierexperimentencommissie NKI
Plesmanlaan 121
1066 CX AMSTERDAM
[redacted]

NETHERLANDS CANCER INSTITUTE
ANTONI VAN LEEUWENHOEK

DEC advies aan CCD

A. Algemene gegevens over de procedure

1. Aanvraagnummer:
2. Titel van het project: Functional analysis of genes implicated in cancer
3. Titel van de NTS: Functionele analyse van genen betrokken bij kanker

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

5. Contactgegevens DEC:
   - naam DEC: DEC NKI
   - telefoonnummer contactpersoon: M. van der Meulen bereikbaar op 020-512 1904
   - e-mailadres contactpersoon: DEC@nki.nl

6. Adviestraject (data dd-mm-jjjj):
   ☒ ontvangen door DEC: 07-12-2016
   ☐ aanvraag compleet
   ☒ in vergadering besproken: 14-12-2016
   ☐ anderszins behandeld
   ☒ termijnonderbreking(en) van 14-12-2016 t/m 11-01-2017
   ☐ besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen
   ☒ aanpassing aanvraag: 11-01-2017
   ☒ advies aan CCD: 20-01-2017

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

8. Eventueel horen van aanvrager:
   - Datum: 14-12-2016
   - Plaats: NKI
   - Aantal aanwezige DEC-leden: 5
   - Aanwezige (namens) aanvrager: [redacted]

9. Correspondentie met de aanvrager
   - Datum: 14 december 2016
   - Gestelde vragen en antwoorden:
   - De aanvrager is verzocht om in het projectvoorstel bij vraag 3.2 duidelijk aan te geven wat de interne samenhang van dit project is. *De tekst is aangepast.*
   - De aanvrager is verzocht om de passage over het maatschappelijk belang bij vraag 3.3 van het projectvoorstel te herschrijven. *De tekst is aangepast.*
   - De aanvrager is verzocht om in de handreiking van begin oktober 2016 zorgvuldig na te gaan of het nodig is om de dieren betrokken bij de verplichte welzijnsmonitoring van de

gegenereerde lijnen onderdeel te maken van dit project. *De aanvrager heeft de aanvraag op dit punt ongewijzigd gelaten. De DEC kan zich vinden in de gehanteerde interpretatie van de handreiking.*
- De aanvrager heeft tal van redactionele suggesties ontvangen. *De suggesties zijn waar nodig adequaat verwerkt in de aanvraag.*

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

B. Beoordeling (adviesvraag en behandeling)

1. Het project is vergunningplichtig (dierproeven in de zin der wet).
2. De aanvraag betreft een nieuwe aanvraag.
3. De DEC is competent om hierover te adviseren.
4. Eén van de DEC-leden is betrokken bij deze projectaanvraag. Dit lid is uitgesloten van de besluitvorming over deze aanvraag.

C. Beoordeling (inhoud)

1. Deze aanvraag heeft een concrete doelstelling en kan getypeerd worden als een project. De opzet komt het best overeen met voorbeeld 4b uit de handreiking 'Invulling definitie project' van de CCD. Het is helder welke handelingen individuele dieren zullen ondergaan. Hierdoor is ook duidelijk welk ongerief individuele dieren zullen ondergaan.
   Het kankeronderzoek, zowel in tumormateriaal van patiënten als in proefdieren, resulteert in de identificatie van grote aantallen genen en genetische elementen die betrokken zijn bij tal van vormen van kanker. De stap die daarop volgt is fundamenteel onderzoek naar de functie van die genen in normale fysiologische omstandigheden. Dit project heeft betrekking op die stap. Er worden genetisch gemodificeerde muizenlijnen gegenereerd (onder een andere vergunning) of geïmporteerd, waarin de door de aanvrager geselecteerde genen veranderd tot expressie komen. Deze muizenlijnen worden in het kader van dit project gefenotypeerd en op basis van de resultaten wordt een besluit genomen over de vraag of verder onderzoek naar de rol van de betreffende genen bij ontstaan en verloop van kanker en naar gerichte therapeutische interventies gerechtvaardigd is. Dit project omvat tevens de welzijnsbewaking van nieuw tot stand gekomen genetisch gemodificeerde lijnen voor zover verwacht wordt dat daarbij sprake zal zijn van een aangetast fenotype. Op basis van ervaring weet de aanvrager dat dit bij maximaal 10% van de lijnen het geval zal zijn.
   Dit onderzoek levert voor alle onderzochte genen de informatie op die nodig is om een "go/no go" beslissing te kunnen nemen over verder onderzoek en om beslissingen te kunnen nemen over het design van dat vervolgonderzoek (waarvoor dan een aparte vergunningaanvraag zal worden ingediend).
   Aangezien verwacht mag worden dat in relatief veel gevallen dit onderzoek tot een "no go" leidt, of tot een aanzienlijke aanpassing van het vervolgonderzoek, is het naar de mening van de DEC gerechtvaardigd om al dit eenvoudige en routinematige preliminaire onderzoek naar de normale fysiologische rol van genen in één aanvraag op te nemen (horizontale benadering). Het indienen van een groot aantal verschillende projectaanvragen voor losse (groepen van) genen, waarin dan ook het vervolgonderzoek naar die genen is opgenomen (verticale benadering), zou er toe leiden

dat in veel projecten al in een vroeg stadium een “no go” beslissing genomen wordt. Ook zou het vervolgonderzoek in deze verticale projectaanvragen noodzakelijkerwijs nog in algemene termen beschreven moeten worden. Met de horizontale benadering wordt voorkomen dat er een groot aantal aanvragen ingediend wordt met in algemene termen beschreven vervolgonderzoek dat mogelijkerwijs uiteindelijk niet zal worden uitgevoerd.
De DEC is er van overtuigd dat de aanvrager gedurende het project op zorgvuldige wijze besluiten zal nemen over de voortgang van het project en dat er niet onnodig dieren gebruikt zullen worden.

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

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

Belangen en waarden

4. Het directe doel van het project is het onderzoeken van de normale functie van genen waarvan gebleken is dat die ontregeld zijn bij kanker. Hiertoe worden modificaties in deze genen geïntroduceerd in muizen, of worden muizen met genetische modificaties geïmporteerd en gefokt, en worden vervolgens de gevolgen van die modificaties voor de normale ontwikkeling van deze muizen geanalyseerd. Deze kennis van de normale functie van de genen is onmisbaar om vast te kunnen stellen wat er fout gaat als die genen, door het feit dat ze ontregeld zijn, bijdragen aan het ontstaan van kanker. Het uiteindelijke doel is om langs deze weg aanknopingspunten te vinden voor therapeutische interventies. Het verband tussen het directe doel en het uiteindelijke doel is weliswaar niet direct, het betreft immers fundamenteel onderzoek, maar wel reëel. Het doel van deze projectaanvraag is gerechtvaardigd binnen de context van het onderzoeksveld, omdat er binnen dat veld consensus is over de waarde van deze aanpak.

5. De belangrijkste belanghebbenden in deze projectaanvraag zijn de proefdieren, de onderzoekers en de doelgroep/patiënten.
Voor de proefdieren geldt dat hun welzijn en integriteit worden aangetast. De dieren zullen beperkt worden in hun natuurlijke gedrag en gedurende de proeven zullen de dieren stress ondervinden en pijn ondergaan. Uiteindelijk zullen ze in het kader van het onderzoek gedood worden. De dieren hebben er belang bij hiervan gevrijwaard te blijven.
Voor de onderzoekers geldt dat ze belangrijke nieuwe wetenschappelijke inzichten kunnen verkrijgen en publiceren, hetgeen vaak de sleutel is tot het verkrijgen van nieuwe onderzoeksmiddelen en -mogelijkheden. Naar de mening van de DEC dient dat echter geen rol te spelen in de ethische afweging over de toelaatbaarheid van het gebruik van proefdieren. Het gaat uiteindelijk om de vraag of dit onderzoek belangrijke maatschappelijke en wetenschappelijke doelen dient (gezondheid, kennis). Dit onderzoek is in de eerste plaats fundamenteel van aard en levert informatie en kennis op die van belang is voor de voortgang van het onderzoek in dit veld.
Voor kankerpatiënten is dit onderzoek van belang, omdat het op termijn kan bijdragen aan een verbetering van de mogelijkheden om kanker te behandelen. Mechanistisch inzicht kan bijdragen aan een gerichte behandeling en diagnostiek met minder bijwerkingen. Dit kan er toe leiden dat de patiënt weer gezond wordt, dan wel een betere kwaliteit van leven heeft. Kunnen beschikken over een breed palet van mogelijke therapieën voor kanker, een ernstige aandoening die zich in een groot aantal vormen manifesteert, is ook van groot belang voor de samenleving.

6. Er is geen sprake van belangwekkende milieueffecten.

Proefopzet en haalbaarheid

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

8. De doelstellingen van het project zijn realistisch en de voorgestelde experimentele opzet en uitkomstparameters sluiten hier logisch bij aan. De DEC is dan ook van mening dat het project goed is opgezet, en dat deze strategie en experimentele aanpak kunnen leiden tot het behalen van de doelstellingen binnen het kader van het project.

Welzijn dieren

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

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

11. Het cumulatieve ongerief als gevolg van de dierproeven is realistisch ingeschat en geclassificeerd. Het ongerief wordt bij het grootste deel van de dieren (73%) bepaald door de weinig ingrijpende handelingen die nodig zijn om de dieren te genotyperen en te fenotyperen. In ca. 10% van de genetische gemodificeerde lijnen die worden onderzocht zal naar verwachting sprake zijn van licht tot maximaal matig ongerief als gevolg van die modificatie. Waar dat te voorzien is wordt, indien mogelijk, gebruik gemaakt van conditionele genetische modificaties. In gevallen waarin het onderzoek aanwijzingen oplevert dat bepaalde genen betrokken kunnen zijn bij weefselhomeostase kan het voor de initiële fenotypering noodzakelijk zijn om weefsels te beschadigen om zo te achterhalen of de betreffende genen een rol spelen bij het herstel. De DEC schat de ernst hiervan in als matig, mede op grond van het feit dat strikte humane eindpunten zullen worden gehanteerd die moeten voorkomen dat het ongerief zich verder ontwikkelt tot ernstig.
    Het cumulatief ongerief voor de dieren is dus juist ingeschat als licht voor 73% van de dieren, matig voor 27% van de dieren.

12. Elke dierproef brengt instrumenteel gebruik van speciaal voor dat doel in gevangenschap gefokte dieren met zich mee, hetgeen op zich al opgevat kan worden als een aantasting van hun integriteit. Omdat dit voor elk project geldt, vermeldt de DEC hier alleen zaken die kenmerkend zijn voor dit specifieke project. De integriteit van de dieren wordt of is aangetast op het niveau van het genoom door het aanbrengen van genetische modificaties, waarna men op zoek gaat naar (vaak subtiele) fenotypische veranderingen die een aanwijzing geven over de normale functie van het gen waarvan de expressie gewijzigd is. Behoudens uitzonderingen, valt niet te

verwachten dat dit tot ingrijpende fenotypische veranderingen leidt. De commissie is daarom van mening dat er sprake is van een beperkte aantasting van de integriteit.

13. Normaal gesproken valt in het grootste deel van dit onderzoek niet te verwachten dat dieren een humaan eindpunt zullen bereiken, omdat de genetische modificatie ernstige gevolgen blijkt te hebben. Het percentage dieren dat naar verwachting een humaan eindpunt zal bereiken is lager dan 1% en is op basis van eerdere ervaringen ingeschat. In veel van die gevallen is de reden om het dier uit de proef te nemen niet gerelateerd aan het experiment of de genetische modificatie, maar algemeen van aard. In bijlage 4 zal naar verwachting wel een aanzienlijk deel van de dieren uit de proef genomen worden, omdat verwacht mag worden dat het ongerief anders hoger dan matig zal worden. De risico's en symptomen die gepaard gaan met de verschillende "challenges" in deze bijlage zijn echter in grote lijnen bekend, zodat adequate monitoring en tijdig ingrijpen mogelijk is. De criteria voor humane eindpunten zijn voldoende specifiek gedefinieerd en toegesneden op de experimenten. De commissie is het eens met de inschattingen en met de gehanteerde humane eindpunten.

3V's

14. De aanvrager heeft voldoende aannemelijk gemaakt dat er geen geschikte vervangingsalternatieven zijn. Meestal zijn de aanwijzingen dat bepaalde genen bij kanker betrokken zouden kunnen zijn afkomstig uit (ex vivo) onderzoek aan tumorweefsel van patiënten en proefdieren. Waar mogelijk wordt de functie van die genen (eerst) in vitro onderzocht. Uiteindelijk vergt echter het onderzoeken van de rol van een gen in de normale ontwikkeling een intact dier waarin de expressie van dat gen is veranderd. Het is niet mogelijk om de vraagstellingen van dit project volledig zonder proefdieren te beantwoorden.

15. Het maximale aantal te gebruiken dieren is realistisch ingeschat en is proportioneel ten opzichte van de gekozen onderzoeksopzet en de looptijd. De onderzoekers hanteren een goede strategie om ervoor te zorgen dat er met het kleinst mogelijk aantal dieren wordt gewerkt waarmee nog een wetenschappelijk betrouwbaar resultaat kan worden verkregen. Door de stapsgewijze aanpak wordt onnodig gebruik van proefdieren voorkomen. De aanvrager heeft inzichtelijk gemaakt hoe en in welke mate dit onderzoek leidt tot fokoverschotten. Het betreft dieren die wel geboren worden in het kader van dit onderzoek, maar niet gebruikt worden in de experimenten (bijvoorbeeld omdat zij niet het juiste genotype hebben). Naar het oordeel van de DEC zijn deze fokoverschotten niet te vermijden.

16. Het project is in overeenstemming met de vereiste van de verfijning van dierproeven. De dieren worden zo kort mogelijk in het experiment gehouden en er worden adequate humane eindpunten gehanteerd. Het gebruik van genetisch gemodificeerde dieren waarbij de genetische modificaties weefselspecifiek en tamoxifen-induceerbaar zijn voorkomt onnodig ongerief door ongecontroleerde tumorgroei. De DEC is ervan overtuigd dat de beschreven proefopzet de meest verfijnde is en dat de dierproeven zo humaan mogelijk worden uitgevoerd.

17. Het project betreft geen wettelijk vereist onderzoek.

Dieren in voorraad gedood en bestemming dieren na afloop proef

18. De aanvrager zal in het project in de regel gebruik maken van zowel mannelijke, als vrouwelijke dieren, tenzij het onderzoek betreft naar genen die een rol spelen bij tumoren die geslachtsgebonden zijn.

19. De dieren zullen in het kader van het project gedood worden. Dit is noodzakelijk om verschillende weefsels en organen na afloop te kunnen uitnemen voor verder onderzoek. De gebruikte dodingsmethode staat vermeld in bijlage IV van richtlijn 2010/63/EU.

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

    NTS

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

D. Ethische afweging

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

2. Voor alle dieren vindt een lichte of matige aantasting van welzijn en integriteit plaats (beschreven in C9 tot C20). De doelstellingen kunnen niet zonder dieren behaald worden. De onderzoekers doen al het mogelijke om het lijden van de dieren en het aantal dieren te beperken.
   Doel van het project is het onderzoeken van de normale functie van genen waarvan gebleken is dat die ontregeld zijn bij kanker. Deze kennis van de normale functie van de genen is onmisbaar om vast te kunnen stellen wat er fout gaat als die genen, door het feit dat ze ontregeld zijn, bijdragen aan het ontstaan van kanker. Het uiteindelijke doel is om langs deze weg aanknopingspunten te vinden voor therapeutische interventies. Er is dringend behoefte aan nieuwe therapeutische benaderingen voor kanker die in combinatie met andere therapieën kunnen worden ingezet om zo de kansen op genezing, of het onder controle houden van de ziekte, te vergroten. Op termijn is het onderzoek daarmee ook voor patiënten en voor de samenleving van belang, omdat het kan bijdragen aan een verbetering van de gezondheid en kwaliteit van leven van veel mensen. De DEC acht het doel van het onderzoek om deze redenen van groot belang.

3. De DEC is overtuigd van het grote belang van de doelstelling van dit project. De commissie is daarnaast overtuigd van de kwaliteit van het onderzoek van de aanvrager. Dit onderzoek is ingebed in een gerenommeerd instituut dat over alle noodzakelijke voorzieningen beschikt. De DEC is van mening dat het project goed is opgezet, en dat de gekozen strategie en experimentele aanpak kunnen leiden tot het behalen van de doelstelling binnen het kader van het project. De aanvrager heeft voldoende aannemelijk gemaakt dat er geen geschikte vervangingsalternatieven zijn, dat het doel niet met minder dieren behaald kan worden, dat de gebruikte aanpak de meest verfijnde is en dat zij zal kunnen voorkomen dat mens, dier en het milieu onbedoelde negatieve effecten ondervinden als gevolg van de dierproeven.
   De DEC is van oordeel dat het hierboven geschetste grote belang van de doelstelling de onvermijdelijke nadelige gevolgen van dit onderzoek voor de dieren, in de vorm van angst, pijn of stress, rechtvaardigt. Aan de eis dat het belang van het onderzoek op dient te wegen tegen het ongerief dat de dieren wordt berokkend, is voldaan.

E. Advies

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

   ☐ De DEC adviseert de vergunning te verlenen onder de volgende voorwaarden
   - Op grond van het wettelijk vereiste (art. 10a1, lid 3) dient de projectleider bij beëindiging van het project een beoordeling achteraf aan te leveren die is afgestemd met de IvD.

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

2. Het uitgebrachte advies is gebaseerd op consensus.

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

Met vriendelijke groet,

[redacted]

ambt. secretaris DEC/NKI

[redacted]

Dierexperimentencommissie NKI
Plesmanlaan 121
1066 CX AMSTERDAM
[redacted]

14 februari 2017

Betreft: Aanvraag AVD301002017840

Geachte CCD,

Hierbij de aangepaste stukken met betrekking tot aanvraag AVD301002017840.
-In de NTS is nu aangegeven dat indien mogelijk dieren van andere instellingen betrokken worden. Wanneer niet dan zal ons eigen instituut deze dieren genereren onder een separate CCD vergunning.
-De humane eindpunten zijn in iedere appendix nader omschreven.
-Vraag L is in iedere appendix ingevuld.
-In iedere appendix is aangegeven dat in het algemeen beide geslachten gebruikt zullen worden, maar dat hiervan afgeweken kan worden om specifiek relevante redenen zoals bijvoorbeeld vrouwelijke dieren in geval van borsttumoronderzoek.

Met vriendelijke groet,

[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.centralecommissiedierproeven.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'.	30100
1.2	Provide the name of the licenced establishment.	Stichting Het Nederlands Kanker Instituut – Antoni van Leeuwenhoek ziekenhuis
1.3	Provide the title of the project.	Functional analysis of genes implicated in cancer

2 Categories

2.1	Please tick each of the following boxes that applies to your project.	x Basic research
		☐ Translational or applied research
		☐ Regulatory use or routine production
		☐ Research into environmental protection in the interest of human or
		☐ 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.

Cancer is caused by malfunctioning of genes regulating cellular processes e.g. cell division, differentiation

and migration. Normally these processes are properly controlled by adequate integration of gene activities in a complex network of signalling pathways. Changes in activity of these genes by mutation, under- or overexpression can result in an imbalanced pathway network leading to aberrant cell behaviour, e.g. uncontrolled proliferation, mobility, and ultimately to cancer. Notably, in tumors of distinct origin often the same pathways are affected by changes in different genes functional in these pathways.

Cancer research over the last decades comprised detailed genomic analyses of tumors from patients and animal models and large numbers of 'in vitro' and 'in vivo' screening approaches. This research has resulted in the identification of a large number of genes and genetic elements controlling them implicated in many different cancer types. However, since genomic analyses often occur at end stage tumor material the identification of these genes as such provides an essential but limited contribution to our understanding how they are involved in the tumorigenic process. For this, thorough functional study of these genes and genetic elements involving both 'in vitro' and 'in vivo' (animal) experiments in normal and oncogenic conditions is required. This yields fundamental knowledge necessary for understanding their possible role in tumor onset, development and progression, which is crucial for translational application in diagnostics and development of therapeutic intervention modalities.

The list of identified cancer genes to date is far from complete and with the still exponential growing potential of genome sequencing, screening and tagging technologies many new genes implicated in cancer development will be identified in the years to come. In this project we will select candidate genes and genetic elements on the basis of published data, e.g. cancer genomic databases from human and other species, and of data from in vitro and in vivo screening and tagging experiments. We want to acquire fundamental knowledge about the normal physiological function of (combination of) these genes and genetic elements by studying the phenotypic effects of controlled (expression) modification at the level of whole animals and at the cellular level, i.e. in GGO mice and cell cultures derived from them, respectively. This will be done in mice carrying the genetic modification in all cells (germ line modifications) or in mice in which the genetic modification is introduced in a tissue and/or temporal specific fashion (conditional modifications). In the latter situation we will also make use of reporter systems that enable to follow the modified cells in the course of development. Already available mouse strains carrying alleles with appropriate modifications will be imported. New strains will be generated in our institute under a separate license.

In general, the functional characterization of selected genes will initially entail the following aspects: viability of germ line modification, Mendelian transmission of the modification, phenotyping of the modified mice, comparison of their behaviour with wild type control mice and thorough molecular and histo-pathological analysis at different ages. In addition, we will derive cell cultures from modified mice to study the effects of the changed gene expression at the molecular and cellular level. The results of these analyses will be leading in the choice from which tissues from the modified mice cell cultures will be derived.

In case germ line modification of selected genes is not viable or certain strong phenotypes preclude the detailed analysis of other, more subtle phenotypes, we will make use of conditional technology enabling temporal and tissue specific gene modification. Examples of early embryo lethality of germ line knockout alleles (see references), which show clear (cancer) phenotypes when alleles are inactivated at adulthood and/or in specific tissues unnoticed in studies of embryo development: pRb, PTEN, APC, Brca1 and BRCA2 (see references below).

References
- Clarke AR, Maandag ER, van Roon M, van der Lugt NM, van der Valk M, Hooper ML, Berns A, te Riele H. Nature 1992, 359: 328-330, Requirement for a functional Rb-1 gene in murine development.
- Di Cristofano A, Pesce B, Cordon-Cardo C, Pandolfi PP. Nat Genet. 1998,19: 348-355, Pten is essential for embryonic development and tumour suppression.
- Oshima M, Oshima H, Kitagawa K, Kobayashi M, Itakura C, Taketo M.
Proc Natl Acad Sci U S A. 1995, 9;92: 4482-4486, Loss of Apc heterozygosity and abnormal tissue building in nascent intestinal polyps in mice carrying a truncated Apc gene.
-Hakem R, de la Pompa JL, Mak TW. J Mammary Gland Biol Neoplasia. 1998 3:431-445, Developmental studies of Brca1 and Brca2 knock-out mice.

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?

The objective of this project is to acquire fundamental knowledge about the normal, physiological function and their role in cellular signaling pathways of specific genes of which the activity is frequently deregulated in cancer development. This knowledge is essential to understand how this deregulation could affect the cross talk with other genetic lesions leading to tumorigenesis. Furthermore, these studies are necessary to predict the consequences of eventual therapeutic intervention strategies specifically targeting the activity of these genes. Moreover, this knowledge will provide essential leads for subsequent research projects for which eventually separate CCD license applications will be assembled. Under this project we will analyze the role of these genes selected on the basis of their data based relevance for tumor development, each in a separate study. In all these gene specific studies we will follow the same routine procedures: phenotyping of the modified mice, thorough histo-pathological and molecular analyses at different ages and characterization of cell cultures derived from these mice.
The results of these studies together with existing data will be leading in designing subsequent 'in vivo' experiments addressing the oncogenic effects of (combinations of) deregulated genes thereby validating their role in human cancers and providing (animal) models for testing therapeutic intervention modalities. These subsequent experiments will be described in additional license applications.
We think we can achieve this objective since our institute holds both the scientific knowledge and technological expertise for these studies: a modern state of the art animal facility including internationally recognized transgenic and imaging facilities embedded in the NWO Roadmap funded MCCA, a well trained staff taking care of animal husbandry, a dedicated animal pathology department with two mouse pathologists supported by a well-equipped pathology lab with all histological technologies available. In addition, all research departments within our institute have access to cell culture facilities, sophisticated digital microscopy (confocal, live-imaging, etc.) and flow cytometry facilities.

3.3 Relevance

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

Scientific: To understand the role of genes identified in large genomic and screening/tagging approaches in the process of cancer onset and progression it is essential to know what the functional role of these genes is in normal development and physiology. This knowledge is also crucial to set up therapeutic

intervention strategies targeting the cellular pathways in which these genes are active.
Social: Cancer has a major negative impact on health / wellbeing of many people. Moreover, the morbidity also has considerable negative economic consequences. There is an urgent need to improve existing treatment strategies since many of them have serious side effects, which limit their full application, since they are often based on targeting (generic) cellular processes also essential for normal healthy cells. Cancer research over the last decades has resulted in the identification of many genes causally implicated in cancer development, thereby providing many possible specific targets for new therapy modalities. The knowledge of the normal function of these genes is essential for the development of targeted therapeutic intervention strategies in evaluating their effectiveness and serious adverse effects. As an illustration of the relevance of these studies see below a short list of publications describing the phenotype of mice carrying modifications in cancer implicated genes and the possible adverse effects as a consequence of therapeutic intervention targeting the their activity.

-Mikkers H, Nawijn M, Allen J, Brouwers C, Verhoeven E, Jonkers J, Berns A. 2004, Mol Cell Biol. 24: 6104-6115, Mice deficient for all PIM kinases display reduced body size and impaired responses to hematopoietic growth factors.
-An N, Kraft AS, Kang Y. 2013, J Hematol Oncol. 6:12, Abnormal hematopoietic phenotypes in Pim kinase triple knockout mice.
-Zhang HW, Ding J, Jin JL, Guo J, Liu JN, Karaplis A, Goltzman D, Miao D. 2010, J Bone Miner Res. 25: 640-652. Defects in mesenchymal stem cell self-renewal and cell fate determination lead to an osteopenic phenotype in Bmi-1 null mice.
-Gu M, Shen L, Bai L, Gao J, Marshall C, Wu T, Ding J, Miao D, Xiao M. 2014, Age 36: 129-139. Heterozygous knockout of the Bmi-1 gene causes an early onset of phenotypes associated with brain aging.

3.4 Research strategy

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

In this project we want to characterize the normal function of cancer related genes in development and physiology at the level of a complete animal. As such the project comprises of many different smaller studies each focusing on a specific gene, gene family or element or set of complementing genes. These studies share the relationship with and relevance for cancer and in practice they follow the same course of straightforward experiments. For a schematic overview of the route each study will follow see flow chart.
Each study starts with the selection of a particular cancer related gene on the basis of existing data indicating their relevance for (human) cancer. For each gene project the following steps will be carried out:

- First we will search literature and depositories for already available mouse strains carrying the gene modification of interest. If available at other laboratories or depositories we will import these mice, if not we will generate the mouse strains in the MCCA facility in our institute and perform the welfare assessment of newly generated GM mice according to the European guidelines. The procedures to generate GM themselves are described in a separate license application from our institute (‘*Generation and cryopreservation of genetically modified mouse strains’*).
  According to the ‘*Herziene Wet op de Dierproeven: het genereren, fokken, genotyperen, monitoren en huisvesten van genetisch gewijzigde dieren’* (Versie: oktober 2016), the welfare assessment of newly

generated genetically modified mouse strains does not require a CCD license upfront, but a license is required for newly genetically modified mouse lines when they exhibit an affected phenotype in their welfare assessment. Beforehand we cannot predict whether the modification of cancer related genes selected for further investigation will lead to an affected phenotype. However, we estimate that up to 10% of the newly generated modified mouse lines might exhibit an affected phenotype and we only apply for a CCD license for this number of mice (appendix 1). The estimation of 10% is based on our own experience and on published data from large phenotyping consortia (Dickinson et al. 2016, Nature 537: 508-514, High-throughput discovery of novel developmental phenotypes).

- In case germ line modifications preclude the analysis of subtle or cell type specific functions at later stages, e.g. in case of embryonic lethality, we will introduce the gene modifications in a temporally controlled and/or tissue/cell type specific fashion. For this we will make use of conditional targeting technology. This approach requires the generation of conditional alleles of the genes of interest and of mouse strains expressing various 'switch' genes enabling controlled activation of gene modifications. Alternatively, 'switch' genes may be introduced somatically (e.g. using viral vectors). These 'switch' genes include sequence specific recombinases (e.g. Cre and Flp) or gene editing systems (e.g. Crispr/Cas). In addition, reporter systems enabling the tracing of cells carrying the modified gene of interest will be incorporated, thereby refining the read out of the experiments. Mouse strains carrying 'switch' alleles and reporter alleles will be referred to as 'tool box' strains. Mouse strains involved in these analyses, i.e. those carrying conditional alleles for the genes of interest, and the 'tool box' strains will either be imported or generated in our transgenic facility. In the latter case, experiments validating their effectiveness will be executed. Validation of these strains and additional technology enabling conditional modification will be described in appendix 2.
- To study the effects of gene modification in all cells of the mouse we will set up cohorts of germ line modified mice and appropriate controls. First we will monitor viability and Mendelian transmission. In case the germ line modification is viable we will follow the modified mice over time and score for aberrant development and behavior. In addition we will perform a thorough molecular and histo-pathological analysis at different ages. In cases of genes that have similar (redundancy, e.g. gene families) or complementary molecular activities we might need to combine modification of gene family members or complementing genes in order to uncover their physiological function. If germ line modification is not viable we will first characterize the phenotype at different stages of gestation. For this we will set up breeding pairs to produce pregnant females carrying modified and control foetuses. In case we want to study gene function at later stages and in specific tissues/cell types we will take the conditional approach and we combine the necessary genetic components to generate the experimental and control cohorts of mice. Decision on timing and tissue/cell type specificity will also be based on the tumor type in which the gene of interest was found to be implicated in addition to their expression profile and other data. After inducing the gene modifications mice will be followed over time and scored for aberrant development and behavior. In addition we will perform a thorough molecular and histo-pathological analysis. In addition to these analyses we will derive cell cultures from modified mice to study the effects of the genetic modification on a cellular and molecular level under strictly defined conditions.

  All these phenotyping and molecular characterization experiments will be described in appendix 3.
- The phenotypic analysis described in appendix 3 will be done on mice kept under standard conditions, not treated in any way. However, existing data from other sources and the results of the analyses described in appendix 3 may indicate that for some genes their function can only be uncovered when

the modified mice or tissues/cells derived from them are exposed to challenging conditions. This might especially be the case when they have a role in stem cell performance supporting tissue homeostasis and regeneration, which as such is also relevant for tumor maintenance. Appendix 4 describes short term pilot experiments involving challenging conditions to obtain the leads for separate, additional license applications that address on a broad scale and in greater depth the function of these genes under specific conditions.

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

The procedures for the generation of genetically modified mouse strains are covered by a separate protocol from the transgenic facility of our institute. Appendix 1 describes the initial welfare assessment of newly generated mouse strains.
The required modifications and technology for conditional induction of genetic modifications will be validated as described in appendix 2 before setting up cohorts for phenotypic characterization of mouse strains carrying modified cancer related genes.
This phenotypic analysis is described in appendix 3 and requires the set up of experimental and control cohorts by conventional breeding procedures. When mice carry conditional modifications, mice will undergo treatments activating the genetic modification of interest. Mice will be closely monitored over time. At increasing ages mice will be sacrificed and total necropsy will be performed. All tissues will be carefully analyzed by molecular and histo-pathological assays. For ' vitro' studies we will also derive cell cultures from the genetically modified mouse strains.
In a minority of cases genetically modified mice or tissues/cells derived from them will be studied in vivo under challenging conditions as described in appendix 4. These experiments address the role of genes primarily involved in tissue regeneration and include transplantation procedures.

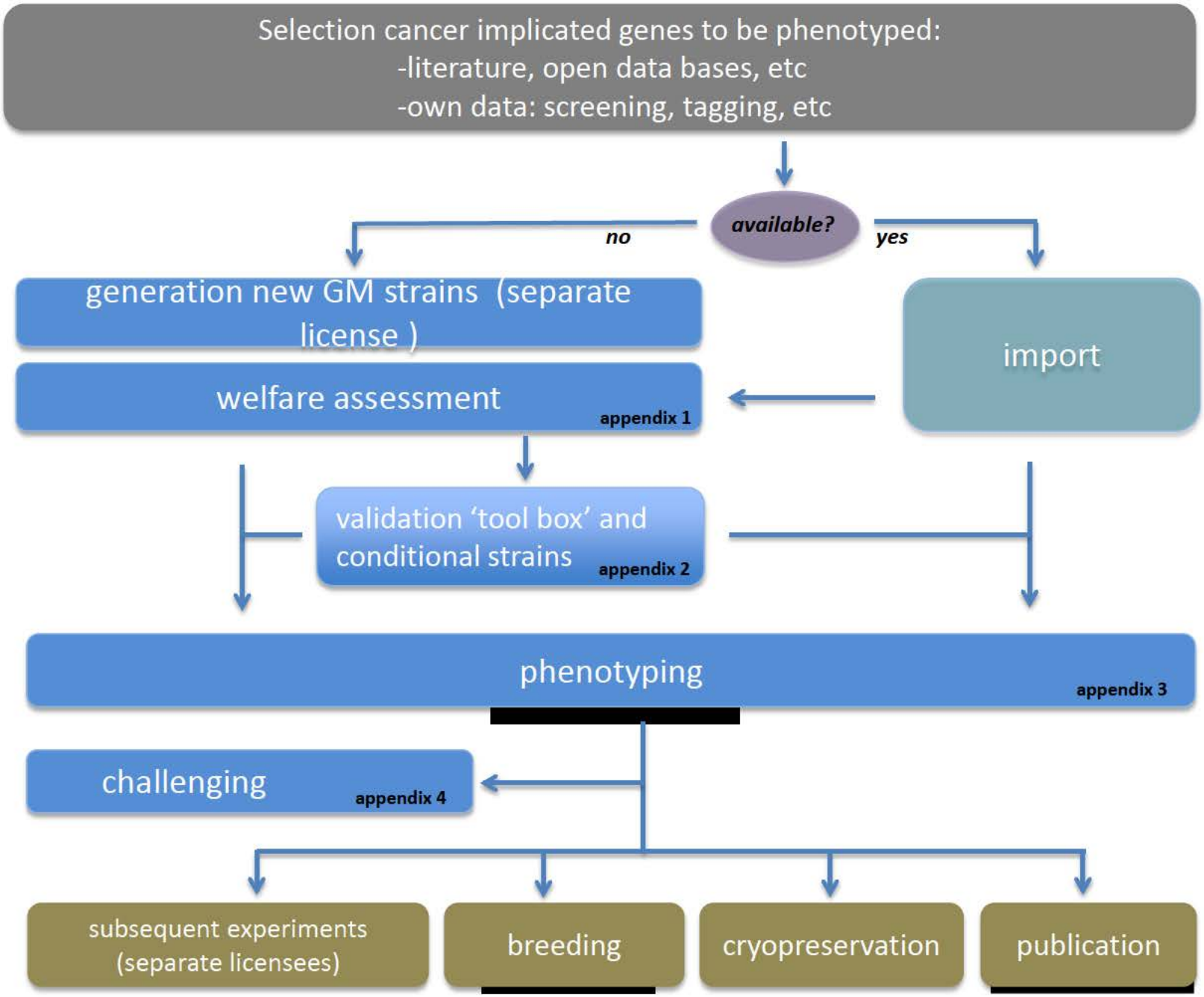
3.4.3 Describe the coherence between the different components and the different steps of the project. If applicable, describe the milestones and selection points.

The flow chart presents an overview of how the project is organized. The coherence of the project is reflected by the fact that each study will take the same approach and consists of a preparation phase and an analytical phase in which the same experimental procedures are followed. In the preparation phase for each study the mouse strains carrying the genetic modification of interest are acquired, their welfare assessed and validated in case of 'tool box' strains (appendices 1 and 2). The analytical phase (appendix 3 and 4) for each separate study follows the same procedure: setting up appropriate cohorts, activation of the modifications if needed, phenotyping at different ages/stages, thorough molecular and histo-pathological characterization and cell culture derivation for ex vivo experiments (appendix 3). In a limited number of these studies the analysis phase will include the characterization of modified mice under challenging conditions (appendix 4). In the analytical phase also cell cultures will be derived from the genetically modified mice.

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	Welfare assessment of new genetically modified mice
2	Validation of conditional genetic modification technology
3	Phenotyping of mice carrying germ line or temporal and/or tissue specific genetic modifications
4	Functional analysis of genetic modifications in mice under challenging conditions

5	
6	
7	
8	
9	
10	


Selection cancer implicated genes to be phenotyped:
-literature, open data bases, etc
-own data: screening, tagging, etc
available?
no
yes
generation new GM strains (separate license)
import
welfare assessment
appendix 1
validation 'tool box' and conditional strains
appendix 2
phenotyping
appendix 3
challenging
appendix 4
subsequent experiments (separate licensees)
breeding
cryopreservation
publication

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.centralecommissiedierproeven.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'.	30100
1.2	Provide the name of the licenced establishment.	Stichting Het Nederlands Kanker Instituut – Antoni van Leeuwenhoek ziekenhuis

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	General welfare assessment of newly generated genetically modified (GM) mice

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 modified (GM) mice will take place via 1) classic transgenesis with DNA injection into zygotes, 2) genome editing technologies (e.g. CRISPR/Cas) in pre-implantation embryo's or 3) injection of genetically modified embryonic stem cells into blastocysts. These procedures will be performed under a separate protocol from our transgenics facility. Basic welfare assessment for the novel (compound) mouse models, for 2 breeding cycles from F2 onwards, is performed according to the guidelines of the new EU directive. Some combination lines will be obtained by conventional crossing of generated lines. New combination lines will be screened for two generations and monitored for spontaneous phenotypic abnormalities.

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

1) Tissue sampling of pups for genotyping and identification:

a. Toe clipping is performed at 5-7 days after birth OR

b. Ear clipping is performed after weaning. In order to improve the precision of the earmarks this is done under anesthesia according to SOP ('pain relief and anesthesia') from the Animal Facility.

The choice between toe- or ear-clip is dependent on the requirements or stage of breeding, as not always

all mice need to be genotyped shortly after birth.
In rare cases genotyping has to be repeated using an additional biopsy; this will be done by tail clipping under anesthesia according to SOP ('pain relief and anesthesia') from the Animal Facility.

2) Welfare assessment:
Mice are monitored daily as part of the standard care by the Animal Facility. In addition the mice will be checked frequently by the researchers for the several parameters as described in the Directive 2010/63/EU: corrigendum of 24 January 2013. The parameters are: appearance, relative size, behavior and posture, growth, coat condition, fertility and mortality figures, and clinical signs. In case of any discomfort more than mild the frequency will be increased.

3) Animals are killed according to SOP ('euthanasia of mice') from the animals facility for instance because they do not have the right genotype or display unacceptable suffering.

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. All techniques are state-of-the-art and are proven effective in modulating inducible gene modification and characterizing GM mice with minimal numbers of animals.

B. The animals

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

Mus musculus: Wild-type and GM mice. Wild-type mice are in part obtained from registered commercial companies; all other mice are obtained from internal breeding programs. Our institute is a license holder with NVWA.
Gender: Both male and female mice will be used, although in some case one gender will generally be selected for gender specific reasons (e.g. to follow mammary gland development).

Numbers: Based on our experience of the last 5 years, for the creation of a new GM mouse line we use on average up to 150 mice (according to the "Besluit Biotechnologie"). However, as the generation of the genetically modified strains per se will be performed under a different protocol from our transgenics facility (MCCA), we do not include these numbers in this current protocol.
The founder generations (F0) obtained from the transgenics facility consist on average of 30 animals per GM line.
For each germ line modification typically three independent founders are selected and initially bred to wild-type (e.g. FVB or B6) mice to create an F1. We will aim to generate substantial numbers of homozygous mice in the next generation (F2). Generations F2 to F4 will undergo an initial welfare assessment. The F4 is included in order to assess the full reproductive cycle over two generations, including gestation in homozygous parents (which does not usually apply to the F2). To have at least 7 males and 7 females for welfare assessment of each generation, we assume at least three litters (average size 7 pups) per generation will need to be generated. Assuming 30 mice for the F0, 30 for the F1 and F2 (to obtain sufficient numbers of male and female homozygous F2 mice), this yields 30 + 3x10 + 3x10 + 3x21 + 3x21 = 222 mice per genetic modification. Based on our experience from the last decade we expect to generate 50 germ line modified mouse lines for which we will need 50 x 222 = 11100 mice for welfare assessment.
For each conditional modification typically 2 independent founders will be selected. For welfare assessment we will need 30 + 2x10 + 2x10 + 2x21 + 2x21 = 154 mice per modification. Based on our

experience from the last decade we expect to generate 25 conditionally modified mouse lines for which we will need 25 x 154 = 3850 mice for welfare assessment.
For each modified mouse line instrumental in the activation and/or tracing of conditional genetic modifications ('tool box' mouse lines) 3 independent founders will be selected. For welfare assessment we will need 30 + 3x10 + 3x10 + 3x21 + 3x21 = 222 mice per modification. Based on our experience from the last decade we expect to generate 25 mouse lines instrumental in conditional genetic modification for which we will need 25 x 222 = 5550 mice for welfare assessment. In total we will need 20500 (11100 + 3850 + 5550) mice for the welfare assessment of 100 new genetically modified mouse lines. However, only for the lines that show an affected phenotype a CCD license is required. We cannot foresee of which lines the phenotype will be affected but we estimate that of up to 10% of the lines this might be the case. This figure is based on our own experience and published data from large phenotyping consortia (Dickinson et al. 2016, Nature 537: 508-514, High-throughput discovery of novel developmental phenotypes). Therefore, we need 0,10 x 20500 = 2050 mice licensed in this appendix.

C. Re-use

Will the animals be re-used?

x No, continue with question D.

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

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

xNo

☐ 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.

- Generation of a new (compound) GM line is carefully considered in advance and should in principle yield new information that can only be obtained by studying the effects of the change of gene activity in a complete animal. In addition, the precise genetic modification should be backed up by solid data and evidence, which can be obtained from a variety of sources, such as genetic screens, in vitro experiments or clinical (large-scale) patient data.
- Before a new GM mouse line is created, we first extensively scan the literature and public resources (EMMA/Infrafrontier, JAX, KOMP and others) for already available GM strains of the same gene or locus. Duplicate mouse strains are not produced.
- The publicly available resource (www.infrafrontier.eu) of genetically modified embryonic stem cells that our transgenics facility has created allows for the generation of compound GM mice without the need for subsequent breeding. This reduces the number of mice obtained by breeding efforts as all modified alleles are already present in the embryonic stem cells (Huijbers et al., Nat Protoc. 2015 Nov;10(11):1755-85. doi: 10.1038).
- The genetic modification will be performed as much as possible in the desired genetic background. This can be achieved by using gene editing technology in pre-implantation embryo's and ES cells from the relevant background. As a result the differences in genetic background of the experimental mice and control mice will be as small as possible thereby increasing the significance and reproducibility of the welfare assessment data.

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

on the environment.

1) All mice are monitored daily for signs of discomfort.
2) There are no negative environmental effects; all mice are housed in controlled environments in individually ventilated cages (IVC). Mice get additional cage enrichment (for instance a mouse home and paper tissues) when signs of distress are observed.

Repetition and duplication

E. Repetition

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

n.a.

Accommodation and care

F. Accommodation and care

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

x No

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

G. Location where the animals procedures are performed

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

x No > Continue with question H.

☐ Yes > Describe this establishment.

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

Classification of discomfort / humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

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

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

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

Anesthesia will be applied during ear or tail clipping according to SOP (‘pain relief and anesthesia’) from the Animal Facility.

I. Other aspects compromising the welfare of the animals

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

The purpose of this appendix is to assess the welfare of mice carrying (conditional) modification in genes

of unknown function. We cannot exclude the occurrence of adverse effects in some of the generated (compound) modified mouse strains. However, based on our 30 years' experience in this type of studies we expect that less than of 10% of the animals the phenotype will be affected and half of these might suffer unexpected discomfort. The nature of the adverse effects and level of discomfort is unpredictable. Rarely, mice are born (the F0 founders) with elephant teeth or showing a severely retarded development, but this also occurs during normal breeding.
In all cases mentioned above the affected animals will be killed immediately in order to limit the discomfort level to moderate.

Explain why these effects may emerge.

In addition to the developmental and physiological consequences of the gene modifications for the handlings of the embryo's or ES cells (e.g. micro-injection or modification per se of ES cells) during the modification procedure might perhaps cause improper development in exceptional cases that emerge in the F0 founders.

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

Mice are monitored daily as part of the standard care by the Animal Facility. In addition the mice will be checked frequently by the researchers for the several parameters as described in the Directive 2010/63/EU: corrigendum of 24 January 2013. The parameters are: appearance, relative size, behavior and posture, growth, coat condition, fertility and mortality figures, and clinical signs. In case of any discomfort more than mild the frequency will be increased.
When discomfort reaches the level of moderate, mice will be euthanized.
Action will be taken on the same day if unexpected adverse effects 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.

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

In case mice are assessed as having a spontaneous discomfort level higher than moderate due to the genetic modification(s) they will not be continued and culled.
We will adhere to the Code of Practice of lab animals used in oncology, in line with internationally agreed rules (see P. Workman et al. Guidelines for welfare and use of animals in cancer research. Br J Cancer 2010; 102: 1555-1577).

In our experiments the most important endpoints that apply are:
-A rapid weight loss of more than 20% of the initial body weight, in case of adult animals. In case of juvenile animals, tailored rules will apply.
-Any sign of tumor formation
-Superficial measurable lesions (by caliper) and/or skin ulceration/necrosis.
-Any abnormal breathing or sign of circulatory problems.
-Any abnormal behavior or locomotion.

Indicate the likely incidence.

Based on our experience and published data (Dickinson et al. 2016, Nature 537: 508-514) only a small fraction (<5%) of genetically modified mouse strains might have spontaneous discomfort higher than mild.

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’).

-GM mice, during welfare assessment: mild (or less) 95%, moderate: <5%

- Mice undergoing ear or tail clips: mild

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

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

The condition of the animals at the end of the experiment will require that the animal is humanely killed.

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.

x Yes

Appendix
Description animal procedures

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- 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'.	30100
1.2	Provide the name of the licenced establishment.	Stichting Het Nederlands Kanker Instituut – Antoni van Leeuwenhoek ziekenhuis

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
		2	Validation of conditional genetic modification technology

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.

In case germ line modifications preclude the analysis of subtle or cell type specific functions at later stages, e.g. in case of embryonic lethality, we will introduce the gene modifications in a temporally controlled and/or tissue/cell specific fashion. For this we will make use of conditional technology. This approach requires the generation of conditional alleles of the genes of interest and of mouse strains expressing various constitutive or inducible 'switch' genes (e.g. recombinases such as Cre, Flp) that activate the genetic modification in conditional genes. Alternative approaches that introduce the 'switch' genes somatically will be involved as well. For example, the use of viral vectors carrying cell type specific promoters driving 'switch' gene expression. In addition, reporter systems enabling the tracing of cells carrying the modified gene of interest will be incorporated thereby refining the read out of the experiments.

Mouse strains carrying 'switch' gene alleles and reporter alleles will be referred to as 'tool box' strains. The functionality of these 'tool box' strains needs to be validated as well as functionality of the conditional modified mouse strains. Conditional genetic modifications should not lead to any phenotype but should serve efficiently as a substrate for 'switch' genes. Validation of these mouse strains and of alternative routes for 'switch' gene introduction will be described in this appendix 2.

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

For the validation of each ‘tool box’ strain, conditional strain or application to introduce ‘switch’ gene systems breeding pairs will be set up in order to obtain the experimental mice with the appropriate genotype. New ‘switch’ gene alleles will be combined with a validated reporter allele and new reporter alleles and new conditional alleles with a validated ‘switch’ gene allele. The validation experiments in case of somatic introduction of ‘switch’ gene systems will be directly done on validated reporter mice. Once the mice with the proper genotypes are available, the following procedures will be performed.

1) Tissue sampling of pups for genotyping and identification

a) toe clipping is performed at 5-7 days after birth OR

b) ear clipping is performed after weaning. This is done under anaesthesia according to SOP. The choice between toe- or ear-clip is dependent on the requirements or stage of breeding, as not always all mice need to be genotyped shortly after birth.

c) tail clipping after weaning under anaesthesia according to SOP (sometimes required to obtain sufficient DNA for careful assessment of the structure of transgene insertions).

2) Overall monitoring of mice for spontaneous phenotypic abnormalities, including regular weighing to monitor growth and possible development of obesity. Appearance, size, growth, behaviour, relative size, breeding parameters and clinical signs will all be assessed.

3) Generation of experimental cohorts in which the necessary genetic elements are combined (e.g. ‘switch’ gene alleles and reporter alleles).

4) Animals are euthanized according to SOP; tissue/cells will be subjected to further molecular and histological characterization directed at the following aspects:

-expression level and tissue/cell type specificity of ‘switch’ gene alleles

-functionality of ‘switch’ genes using validated reporter alleles as substrates

-inducibility of ‘switch’ genes using validated reporter alleles

-functionality of somatic delivery of switch genes using validated reporter alleles

-functionality of reporter alleles using validated ‘switch’ gene alleles

-functionality of conditional alleles

5) In case of inducible gene modification animals will be exposed to the appropriate inducing agents or control substances by one or more of the following routes (the best administration route and schedule is determined by the physicochemical nature/pharmacological properties of the inducing agent and the required extent and timing of the intended deletion):

a) in diet or drinking water, for maximally 2 weeks

b) intra-peritoneal injection (maximally 5 times)

c) oral administration (maximally 5 times)

d) topical application on the skin under anaesthesia according to SOP (maximally 3x)

6) For somatic introduction of gene modifications, various formulations of ‘switch’ gene systems can be used e.g. viral vector suspensions, liposome suspensions and DNA, RNA and proteins formulated in various solvents. In case the introduction of these systems will raise an immune response against the cells that have been targeted (e.g. viral vectors generating foreign antigens) we will supplement the drinking water of the mice with immune suppressants. For somatic introduction of the ‘switch’ gene systems, mice will be subjected to either one of the following procedures:

a) injection either sub-cutaneous, intra-peritoneal, intra-muscular, intra-venous, intra-thoracic; if necessary under anaesthesia according to SOP (1x)

b) introduction under anaesthesia according to SOP without surgery via either one of the openings in the

body, e.g. anal, urine tract, vaginal, intra-tracheal, inhalation, oral, milk duct mammary gland (1x)
c) surgical intra-cranial installation under anaesthesia and analgesia according to SOP (1x)
d) tattoo of the skin under anaesthesia according to SOP (maximally 3x).
e) shaving of the skin and topical application (ointment) (maximally 3x)
7) Blood sampling according to SOP at different time points after activation of the genetic modification.

None of the listed procedures causes a discomfort level more than 'mild' except for the surgical intra-cranial installation. Only a very small number of mice will be involved in this procedure (< 0,5%)

Welfare assessment:
Mice are monitored daily as part of the standard care by the Animal Facility. In addition the mice will be checked frequently by the researchers for the several parameters as described in the Directive 2010/63/EU: corrigendum of 24 January 2013. The parameters are: appearance, relative size, behaviour and posture, growth, coat condition, fertility and mortality figures, and clinical signs. In case of any discomfort more than mild the frequency will be increased.

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. All techniques are state-of-the-art and are proven effective in modulating inducible gene modification and characterizing GM mice with minimal numbers of animals.

B. The animals

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

Mus musculus: Wild-type and GM mice. Wild-type mice are in part obtained from registered commercial companies; all other mice are obtained from internal breeding programs. The institute is a license holder with NVWA.
Gender: Both male and female mice will be used, although in some case one gender will be preferred for gender specific reasons (e.g. to validate mammary gland specific expression).

We expect to functionally validate 25 new 'tool box' strains (10 carrying constitutive active 'switch' gene alleles, 10 inducible active 'switch' gene alleles and 5 new reporter alleles) and 25 new conditional alleles. In addition, based on the experiments during the last 5 years during which a broad range of conditional technologies has been applied, we fore see to analyse the effectiveness of 25 new strategies to somatically introduce 'switch' gene systems.
For functional analysis of new 'switch' alleles we have to set up groups of mice in which new 'switch' alleles are combined with validated 'reporter' alleles. This will be done by cross breeding which involves more mice due to Mendelian transmission (for each experimental mouse on average 4 mice are needed excluding parents)
To validate inducibility of 'switch' alleles we will test on average 5 conditions and the read out will be done using reporter mice as described above.
For validation of new 'reporter' alleles we have to set up groups of mice in which new 'reporter' alleles are combined with validated 'switch' alleles. This will be done by cross breeding which involves more mice due to Mendelian transmission (for each experimental mouse on average 4 mice are needed excluding parents).

For each new application to somatically introduce ‘switch’ alleles (e.g. viral) we will use on average 5 variables. For these experiments we will use validated ‘reporter’ strains. The nature of the variables depends on the application (e.g. dosage).
To validate the functionality of conditional alleles we will combine the new conditional allele with validated ‘switch alleles’. This will be done by cross breeding which involves more mice due to Mendelian transmission (for each experimental mouse on average 4 mice are needed excluding parents).

According to the *‘Herziene Wet op de Dierproeven: het genereren, fokken, genotyperen, monitoren en huisvesten van genetisch gewijzigde dieren’* (Versie: oktober 2016), a CCD license approval is needed only for experimental mice. Mice necessary for breeding do not have to be included.
The numbers of experimental and breeding mice involved in these experiments are listed below. The number of experimental mice are indicated in roman (and the number of additional breeding mice in italics).
The numbers are based on a group size of 5 animals for all of these analyses as in our experience this number is sufficient to draw firm conclusions.

nr mice needed	analysis		breeding	
Tool box lines				
Constitutive activity				
Expression analysis	5		*15*	
Functional analysis:	5		*30*	
Total per line	10		*45*	
3 lines per modification	30		*135*	
10 modifications		300		*1350*
Inducible activity				
Expression analysis	5		*15*	
Functional analysis (5 conditions)	25		*120*	
Total per line	30		*135*	
3 lines per modification	90		*405*	
10 modifications		900		*4050*
Reporters				
Expression analysis	5		*15*	
Functional analysis:	5		*30*	
Total per line	10		*45*	
3 lines per modification	30		*135*	
5 reporter lines		150		*625*
Somatic 'switch' gene introduction				
5 variables (e.g. dosage) + control	30			
25 applications		750		
Conditional alleles				
Recombination validation	5		*30*	
2 lines per cond. allele	10		*60*	
25 cond. alleles		250		*1500*
Total experimental:	2350			
Breeding				*7525*
Grand total:	9875			
Licensed in this application: 2350				

C. Re-use

Will the animals be re-used?

x No, continue with question D.

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

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

x 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.

- Generation and testing of a new 'tool box' and conditional lines is carefully considered in advance and should in principle yield new possibilities to study the effects of changes in gene activity in a complete animal.
- Before a new 'tool box' or conditional mouse line is created and tested we first extensively scan the literature and public resources (EMMA/Infrafrontier, JAX, KOMP and others) for already available 'tool box' strains and conditional strains of the same gene or locus. Duplicate mouse strains are not produced.

  In addition we will search the literature and other resources for the most efficient technologies for induction and somatic delivery.

  The publicly available resource (www.infrafrontier.eu) of genetically modified embryonic stem cells that our transgenics facility has created allows for the generation of compound GM mice without the need for subsequent breeding. This reduces the number of mice obtained by breeding efforts as all modified alleles are already present in the embryonic stem cells (Huijbers et al., Nat Protoc. 2015 Nov;10(11):1755-85. doi: 10.1038).
- The genetic modification will be performed as much as possible in the desired genetic background in which the subsequent experiments in this application (appendix 3 and 4) will be performed. This can be achieved by using gene editing technology in pre-implantation embryos and ES cells from the relevant background.

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

1) All mice are monitored daily for signs of discomfort.

2) There are no negative environmental effects; all mice are housed in controlled environments in individually ventilated cages (IVC). Mice get additional cage enrichment (for instance a mouse home and paper tissues) when housed solitarily (while minimized as much as possible, this is sometimes required during the genotype screening phase) and also when signs of distress are observed. In these cases monitoring will be intensified.

Repetition and duplication

E. Repetition

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

n.a.

Accommodation and care

F. Accommodation and care

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

x No

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

G. Location where the animals procedures are performed

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

x No > Continue with question H.

☐ Yes > Describe this establishment.

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

Classification of discomfort / humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

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

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

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

Anaesthesia according to SOP will be applied
-during ear or tail clipping,
-injection either intra-muscular, intra-thoracic.
-introduction via either one of the openings in the body, e.g. anal, urine tract, vaginal, intra-tracheal, inhalation, oral, milk duct mammary gland
-tattoo of the skin
Anaesthesia and analgesia will be applied according SOP
-during and after surgical intra-cranial installation

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 more adverse effects during cross-breeding of 'tool box' and conditional modified strains in the context of their validation.

Explain why these effects may emerge.

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

The mice are monitored daily. Action will be taken on the same day if unexpected adverse effects 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.

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

In case mice are assessed as having a spontaneous discomfort level higher than mild due to the genetic modification(s) they will not be continued and culled except for the mice that intracranially injected mice (< 1%) that might suffer moderate discomfort.

We will adhere to the Code of Practice of lab animals used in oncology, in line with internationally agreed rules (see P. Workman et al. Guidelines for welfare and use of animals in cancer research. Br J Cancer 2010; 102: 1555-1577).

In our experiments the most important endpoints that apply are:
-A rapid weight loss of more than 20% of the initial body weight, in case of adult animals. In case of juvenile animals, tailored rules will apply.
-Any sign of tumor formation
-Any skin defects
-Any abnormal breathing or sign of circulatory problems.
-Any abnormal behavior or locomotion.
-In case of intracranial installation: any clinical symptoms of discomfort exceeding the level of moderate discomfort to be expected due to the intracranial installation procedure.

Indicate the likely incidence.

Based on our experience so far the likelihood of this happening is low (only a very small fraction (<1%) of 'tool box' or conditional mouse strains).

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').

None of the mice that have undergone one of the listed procedures will suffer discomfort at a level more than 'mild' except for the surgical intra-cranial installation, which causes an estimated moderate discomfort. Only a very small minority of the mice will be involved in this procedure (< 0,5%).

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

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

The condition of the animals at the end of the experiment will require that the animal is humanely killed.

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.

x Yes

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.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'.	30100
1.2	Provide the name of the licenced establishment.	Stichting Het Nederlands Kanker Instituut – Antoni van Leeuwenhoek ziekenhuis

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
3	Phenotyping of mice carrying germ line or temporal and/or tissue specific genetic modifications

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.

To study the effects of gene modification in all cells of the mouse we will set up cohorts of germ line modified mice and appropriate controls. First we will monitor viability and Mendelian transmission. In case the germ line modification is viable we will follow the modified mice over time and monitor development and behaviour. In addition we will perform a thorough molecular and histo-pathological analysis at different ages. In cases of families of genes that have similar molecular activities (redundancy) or sets of complementing genes we might need to combine modification of multiple genes in order to uncover their physiological function. If germ line modification is not viable we will first characterize the phenotype at different stages of gestation. For this we will set up breeding pairs to produce pregnant females carrying modified and control foetuses.

In case we want to study gene function at later stages and in specific tissues/cell types we will take the conditional approach and combine the necessary genetic components to generate the experimental and control cohorts of mice by efficient breeding strategies. These genetic components include conditional alleles of the genes of interest and of mouse strains expressing various constitutive or inducible 'switch' genes (e.g. recombinases such as Cre, Flp) that activate the genetic modification in conditional genes.

Alternative approaches that introduce the 'switch' genes somatically will be involved as well. For example, the use of viral vectors carrying cell type specific promoters driving 'switch' gene expression. In addition, reporter systems enabling the tracing of cells carrying the modified gene of interest will be incorporated thereby refining the read out of the experiments.
Decision on timing and tissue/cell type specificity will be primarily based on the tumor type in which the gene of interest was found to be implicated in addition to their expression profile and other data. After inducing the gene modifications we will follow the mice over time and monitor development and behaviour. In addition we will perform a thorough molecular and histo-pathological analysis at on average 2 time points based on the expression and functional characteristics of the genetic modification of interest.

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

For the generation of the genetically modified and control cohorts breeding pairs will be set up in order to obtain the experimental mice with the appropriate genotype. For this the following procedures will be necessary:
1) Tissue sampling of pups for genotyping and identification
a) toe clipping performed at 5-7 days after birth OR
b) ear clipping performed after weaning. This is done under anesthesia according to SOP. The choice between toe- or ear-clip is dependent on the requirements or stage of breeding, as not always all mice need to be genotyped shortly after birth.
c) tail clipping after weaning under anesthesia according to SOP (sometimes required to obtain sufficient DNA for careful assessment of the structure of transgene insertions).
2) In case of inducible gene modification animals will be exposed to the appropriate inducing 'switch' gene activation agents or control substances by one or more of the following routes (the best administration route and schedule is determined by the physicochemical nature/pharmacological properties of the inducing agent and the required extent and timing of the intended deletion):
a) in diet or drinking water, for maximally 2 weeks
b) intra-peritoneal injection (maximally 5 times)
c) oral administration (maximally 5 times)
d) topical application on the skin under anesthesia according to SOP (maximally 3x).
3) For somatic introduction of gene modifications, various formulations of 'switch' gene systems can be used e.g. viral vector suspensions, liposome suspensions and DNA, RNA and proteins formulated in various solvents. In case the introduction of these systems will raise an immune response against the cells that have been targeted (e.g. viral vectors generating foreign antigens) we will supplement the drinking water of the mice with immune suppressants. For somatic introduction of the conditional gene, mice will be subjected to either one of the following procedures:
a) injection either sub-cutaneous, intra-peritoneal, intra-muscular, intra-venous, intra-thoracic; if necessary under anesthesia according to SOP (1x)
b) introduction under anesthesia according to without surgery via either one of the openings in the body, e.g. anal, urine tract, vaginal, intra-tracheal, inhalation, oral, milk duct mammary gland (1x)
c) surgical intra-cranial installation under anesthesia and analgesia according to SOP (1x)
d) tattoo of the skin under anesthesia according to (maximally 3x)
e) shaving of the skin and topical application (ointment) (maximally 3x)
Once the cohorts of mice carrying the relevant genetic modifications have been set up, either with or

without (somatic) induction, the following procedures will be performed:
4) Overall monitoring of mice for spontaneous phenotypic abnormalities, including regular weighing to monitor growth and possible development of obesity. Appearance, size, growth, behavior, relative size, breeding parameters and clinical signs will all be assessed.
5) Animals are killed according to SOP; tissue/cells will be harvested and for subjected to the following procedures:
-molecular characterization directed at the following aspects:

- reporter allele expression
- presence of gene modification of interest

-histo-pathology,
-tissue culture derivation
In some cases depending on the expression and functional characteristics of the genetic modification of interest, the following additional procedures will be performed:
6) Blood sampling according to SOP at different time points after activation of the genetic modification to measure a wide range of relevant blood parameters.
7) Mice are injected with DNA labeling substances (e.g. BrdU) before short before sacrifice in order to detect proliferating cells by histo-chemistry

None of the listed procedures causes a discomfort level more than 'mild' except for the surgical intra-cranial installation, which causes an estimated moderate discomfort. Only a very small minority of the mice will be involved in this procedure (< 0,5%).

Welfare assessment:
Mice are monitored daily as part of the standard care by the Animal Facility. In addition the mice will be checked frequently by the researchers for the several parameters as described in the Directive 2010/63/EU: corrigendum of 24 January 2013. The parameters are: appearance, relative size, behavior and posture, growth, coat condition, fertility and mortality figures, and clinical signs. In case of any discomfort more than mild the frequency will be increased.

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. All techniques are state-of-the-art and are proven effective in modulating inducible gene modification and characterizing GM mice with minimal numbers of animals.

B. The animals

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

Mus musculus: Wild-type and GM mice. Wild-type mice are in part obtained from registered commercial companies; all other mice are obtained from internal breeding programs. Our institute is a license holder with NVWA.
Gender: Both male and female mice will be used, although in some case one gender will generally be selected for gender specific reasons (e.g. to study mammary gland development).

We expect to characterize 50 new lines carrying germ line modifications. Most of the modification will be homozygous. Controls for these experimental mice will be wild type littermates.
We expect to generate 5 compound modified mouse lines carrying modified redundant or complementing

alleles. Generating the experimental mice will take significantly more mice in order to breed two modified alleles to homozygosity.
For 25 genes we expect to study their effects of conditional modification under, on average, 2 conditions e.g. in a tissue specific and/or temporally controlled fashion. In these cases we will incorporate reporter alleles to trace the cells that have undergone the modification, which will need more mice for the generation of the experimental cohorts.
For 10 genes we expect to study their effects of conditional modification after somatic introduction of 'switch' genes. For these analyses we will also make use of reporter alleles to trace the cells that have undergone the modification. On average we expect to use 2 applications of 'switch' gene delivery.
Cohorts of experimental mice in which multiple modified alleles are combined will be generated by cross breeding. This will involve a considerable number of mice.
However, according to the *'Herziene Wet op de Dierproeven: het genereren, fokken, genotyperen, monitoren en huisvesten van genetisch gewijzigde dieren'* (Versie: oktober 2016), a CCD license approval is needed only for experimental mice. Mice necessary for breeding do not have to be included.
The numbers of experimental and breeding mice in this appendix are listed below. The number of experimental mice are indicated in roman (and the number of additional breeding mice in italics).
These numbers are based on a group size of 10 experimental and 10 control animals for all of these analyses as in our experience this number is sufficient to draw firm conclusions.

nr mice needed	experimental + controls		breeding	
Germline modification				
per line 1 time point	20		*24*	
3 time points	60		*72*	
2 lines each	120		*144*	
50 modified alleles		6000		*7200*
Compound germline modification				
per compound modified line	20		*96*	
3 time points	60		*288*	
10 compound modified lines		600		*2880*
Conditional modification				
per line + reporter	20		*48*	
2 time points	40		*96*	
2 'switch' alleles per line	80		*192*	
25 lines		2000		*4800*
Somatic 'switch' gene introduction				
per line + reporter	20		*48*	
2 applications	40		*96*	
10 lines		400		*960*
Total experimental	9000			
breeding				*15840*

Grand total: 24840
Licensed in this application: 9000

C. Re-use

Will the animals be re-used?

x No, continue with question D.

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

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

x 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.

- Generation and characterization of a new lines carrying modification in cancer related genes is carefully considered in advance and should in principle yield functional information that can not be obtained by 'in vitro' studies but only in a complete animal.
- Before a new mouse line is created and phenotyped we first extensively scan the literature and public resources (EMMA/Infrafrontier, JAX, KOMP and others) for already existing data on the function of the same gene or locus. We will not duplicate the generation of mouse strains.
  The publicly available resource (www.infrafrontier.eu) of genetically modified embryonic stem cells that our transgenics facility has created allows for the generation of compound GM mice without the need for subsequent breeding. This reduces the number of mice obtained by breeding efforts as all modified alleles are already present in the embryonic stem cells (Huijbers et al., Nat Protoc. 2015 Nov;10(11):1755-85. doi: 10.1038).
- The genetic modification will be performed as much as possible in the desired genetic background in which the experiments in this appendix will be performed. This can be achieved by using gene editing technology in pre-implantation embryos and ES cells from the relevant background.

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

1) All mice are monitored daily for signs of discomfort.
2) There are no negative environmental effects; all mice are housed in controlled environments in individually ventilated cages (IVC). Mice get additional cage enrichment (for instance a mouse home and paper tissues) when housed solitarily (while minimized as much as possible, this is sometimes required during the genotype screening phase) and also when signs of distress are observed. In these cases monitoring will be intensified.

Repetition and duplication

E. Repetition

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

n.a.

Accommodation and care

F. Accommodation and care

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

x No

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

G. Location where the animals procedures are performed

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

x No > Continue with question H.

☐ Yes > Describe this establishment.

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

Classification of discomfort / humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

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

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

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

Anaesthesia with isoflurane will be applied
-during ear or tail clipping,
-injection either intra-muscular, intra-thoracic.
-introduction surgery via either one of the openings in the body, e.g. anal, urine tract, vaginal, intra-tracheal, inhalation, oral, milk duct mammary gland
-tattoo of the skin
Anaesthesia and analgesia will be applied according SOP
-during and after surgical intra-cranial installation

I. Other aspects compromising the welfare of the animals

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

The purpose of this appendix is to study phenotypic consequences of (conditional) gene modification in living mice. Since the physiological function of the genes of interest is not known and in fact the aim of these studies and we cannot exclude the occurrence of adverse effects in some of the generated (compound) modified mouse strains. However, based on our 30 years' experience in this type of studies we expect that less than of 10% of the animals the phenotype will be affected and half of these might suffer unexpected discomfort. The nature of the adverse effects and level of discomfort is unpredictable. Rarely, mice are born (the F0 founders) with elephant teeth or showing a severely retarded development, but this also occurs during normal breeding.
In all cases mentioned above the affected animals will be killed immediately in order to limit the discomfort level to moderate

Explain why these effects may emerge.

In addition to the developmental and physiological consequences of the gene modifications for the handlings of the embryo's or ES cells (e.g. micro-injection or modification per se of ES cells) during the modification procedure might perhaps cause improper development in exceptional cases that emerge in the F0 founders.

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

The mice are monitored daily. Action will be taken on the same day if unexpected adverse effects 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.

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

In case mice are assessed as having a spontaneous discomfort level higher than moderate due to the genetic modification(s) they will not be continued and culled.
We will adhere to the Code of Practice of lab animals used in oncology, in line with internationally agreed rules (see P. Workman et al. Guidelines for welfare and use of animals in cancer research. Br J Cancer 2010; 102: 1555-1577).

In our experiments the most important endpoints that apply are:
-A rapid weight loss of more than 20% of the initial body weight, in case of adult animals. In case of juvenile animals, tailored rules will apply.
-Any sign of tumor formation
-Superficial measurable lesions (by caliper) and/or skin ulceration/necrosis.
-Any abnormal breathing.
-Any abnormal behavior.

Indicate the likely incidence.

Based on our experience and published data (Dickinson et al. 2016, Nature 537: 508-514) only a small fraction (<5%) of genetically modified mouse strains might have spontaneous discomfort higher than mild.

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').

None of the mice that have undergone one of the listed procedures will suffer discomfort at a level more than 'mild' except for the surgical intra-cranial installation, which causes an estimated moderate discomfort. Only a very small number of the mice will be involved in this procedure (< 1%).

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

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

The condition of the animals at the end of the experiment will require that the animal is humanely killed.
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.
x Yes

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.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'.	30100
1.2	Provide the name of the licenced establishment.	Stichting Het Nederlands Kanker Instituut – Antoni van Leeuwenhoek ziekenhuis

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
4	Functional analysis of genetic modifications in mice under challenging conditions

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.

For some genes the results of the analyses described in appendix 3 and existing data from other sources may indicate that their function can only be uncovered when the modified mice or tissues/cells derived from them are exposed to challenging conditions. This might be the case when they have a role is in stem cell performance supporting tissue homeostasis and regeneration. Such a role could be very relevant for tumor maintenance and post-treatment relapses. This appendix 4 describes short term (pilot) experiments involving challenging conditions to obtain the leads for separate, additional licence applications that address on a broad scale and in greater depth the function of these genes.
The conditions we want to apply enable to test in a defined and reproducible fashion the ability of genetically modified tissues/cells to contribute to the restoration of damaged tissues. This approach is especially suited to address the role of genes in (tissue) stem cell function. The tissues in which we intend to perform these analyses include the skin, mammary gland, the hematopoietic system and the liver. The research in our institute has a strong focus on tumorigenesis in these tissues, which are also most suitable for the analysis of stem cell performance, since these tissues have an intrinsically high regeneration potential.
We will perform these analyses only for genes for which we have indications that they are involved in

tissue homeostasis and regeneration and the regular approach described in appendix 3 is not informative.

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

Two types of animal experiments will be carried out:
1) Experiments in which genetically modified animals undergo tissue damaging procedures and in which restoration of these tissues will be analysed at different time points directly.
2) Experiments in which recipient animals undergo tissue damaging procedures and subsequently serve as acceptors for tissues/cells transplantations from genetically modified mice. The performance of these grafts in the recipient mice will be analysed, e.g. their ability to tissue restoration, at different time points.

For the type 1 experiments, we will perform the following tissue damaging procedures in experimental mice directly:
1) total body irradiation according to SOP
2) targeted, local irradiation to damage specific tissues according to SOP
3) local exposure to chemical agents (e.g. naphtaline damaging lung tissue); the method of application is determined by the physicochemical nature/pharmacological properties of the damaging agent
4) skin wounding under anaesthesia and analgesia according to SOP
5) partial mammary fat pad clearance under anaesthesia and analgesia according to SOP
6) partial hepatectomy under anaesthesia and analgesia according to SOP
In some cases using conditionally modified mice also carrying inducible 'switch' alleles the gene modification will be activated after the tissue damaging procedure. For this, animals will be exposed to the appropriate inducing 'switch' gene activation agents or control substances by one or more of the following routes (the best administration route and schedule is determined by the physicochemical nature/pharmacological properties of the inducing agent and the required extent and timing of the intended deletion):
a) in diet or drinking water, for maximally 2 weeks
b) intra-peritoneal injection (maximally 5 times)
c) oral administration (maximally 5 times)
d) topical application on the skin under isoflurane anesthesia (maximally 3x).

For the type 2 experiments, we will perform the following tissue damaging and transplantation procedures in recipient mice:
1) total body irradiation to deplete the hematopoietic system of the recipient mice followed by transplantation by intra-venous injection of bone marrow cell suspensions from genetically modified and control mice
2) surgical removal of small piece of the dorsal skin (wounding) of the recipient mice followed by transplantation skin tissue or skin cell suspensions from genetically modified and control mice under anaesthesia and analgesia in the wounded skin of recipient mice
3) mammary fat pad clearance of recipient mice and transfer of mammary gland tissue or mammary cell suspensions from genetically modified or control mice in cleared fat pads of recipient mice under anaesthesia and analgesia.
Transplanted tissues or cells may carry reporter alleles in order to accurately distinguish between

transplanted and host tissues/cells.
In some cases the gene modification in the donor tissue will may be activated after transplantation of tissues from conditionally modified mice carrying inducible 'switch' alleles. For this, after grafting the recipient animals will be exposed to the appropriate inducing 'switch' gene activation agents or control substances by one or more of the following routes (the best administration route and schedule is determined by the physicochemical nature/pharmacological properties of the inducing agent and the required extent and timing of the intended deletion):
a) in diet or drinking water, for maximally 2 weeks
b) intra-peritoneal injection (maximally 5 times)
c) oral administration (maximally 5 times)
d) topical application on the skin under isoflurane anesthesia (maximally 3x).

Once the cohorts of recipient mice carrying the relevant tissue damage have been set up, either with or without tissue/cell suspension transplantation, the following procedures will be performed:
-assessment of regeneration of the affected tissue by histopathology
-overall monitoring of mice for spontaneous phenotypic abnormalities, including regular weighing to monitor growth and possible development of obesity. Appearance, size, growth, behavior, relative size and clinical signs will all be assessed.
-i.p. injection with DNA labeling substances (e.g. BrdU) before sacrifice in order to detect proliferating cells
-animals are killed according to SOP; tissue/cells will be harvested and for subjected to the following procedures:

- molecular characterization directed at the following aspects:
    - reporter allele expression
    - presence of gene modification of interest
- histo-pathology,
- tissue culture derivation

Welfare assessment:
Mice are monitored daily as part of the standard care by the Animal Facility. In addition the mice will be checked frequently by the researchers for the several parameters as described in the Directive 2010/63/EU: corrigendum of 24 January 2013. The parameters are: appearance, relative size, behavior and posture, growth, coat condition, fertility and mortality figures, and clinical signs. In case of any discomfort more than mild the frequency will be increased.

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. All techniques are state-of-the-art and are proven effective in modulating inducible gene modification and characterizing GM mice with minimal numbers of animals.

B. The animals

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

Mus musculus: Wild-type and GM mice. Wild-type mice are in part obtained from registered commercial companies; all other mice are obtained from internal breeding programs. Our institute is a license holder with NVWA.

All mice will be enrolled as adult.
Gender: Both male and female mice will be used, although in some case one gender will generally be selected for gender specific reasons (e.g. to study mammary gland development).

We expect to characterize the effects of 30 genetic modifications in type 1 experiments: 13 in mice after damaging by irradiation, 5 after tissue damaging by agents, 5 after skin wounding, 5 after partial mammary fat pad clearance and 2 after hepatectomy.
Per experiment we will use 10 experimental and 10 wild type control mice as in our experience these numbers are sufficient to draw significant conclusions.
In type 2 experiments we expect to analyze the transplants from 18 GM lines: 6 after bone marrow transplantation, 6 after skin/cell transplantation and 6 after mammary tissue/cell transplantation. For these transplantation experiments isogenic recipients will be used in order to immunologically match donor and recipient and to maximize the take rate of the transplants.
Per experiment we will use 10 recipients for transplantation of experimental tissue/cells and 10 recipients for transplantation of control tissue/cells as in our experience these numbers are sufficient to draw significant conclusions.

According to the *'Herziene Wet op de Dierproeven: het genereren, fokken, genotyperen, monitoren en huisvesten van genetisch gewijzigde dieren'* (Versie: oktober 2016), a CCD license approval is needed only for experimental mice. Mice necessary for breeding do not have to be included.
The numbers of experimental and breeding mice involved in these experiments are listed below. The number of experimental mice are indicated in roman (and the number of additional breeding mice in italics).

nr mice needed	experimental + controls		*breeding*	
Type1 experiment				
per GM line 1 time point	20		*72*	
3 time points	60		*216*	
30 GM lines		1800		*6480*
Type 2 experiment				
per GM tissue/cell transplant	20		*96*	
3 time points	60		*288*	
transplants from 18 GM lines		1080		*5184*
Total experimental		2880		
breeding				*11664*
Grand total:	14544			
Licensed in this application:	**2880**			

C. Re-use

Will the animals be re-used?

x No, continue with question D.

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

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

x 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 experiments to address the involvement of genes in stem cell performance and tissue regeneration using genetically modified mouse lines are carefully considered in advance and should in principle yield functional information that cannot be obtained by 'in vitro' studies but only in a complete animal. We will perform these experiments only for genes for which data from literature or our own results (e.g. from experiments described in appendix 3) strongly indicate a role in stem cell performance and tissue regeneration.
- All transplanted tissues or cells will carry reporter alleles in order to accurately distinguish between transplanted and host tissues/cells. This increases the sensitivity of the analyses and reduces the number of mice necessary for drawing sound conclusions
- In case of transplantation assays, if possible we will use recipient mice for both experimental and control transplant (e.g. skin transplantations on the left and right dorsal site). This improves the experimental setting and reduces the number of mice needed without increasing discomfort.
- The genetic modifications have been introduced in mice as much as possible in the desired genetic background in which the experiments in this appendix will be performed. This has been achieved by using gene editing technology in pre-implantation embryo's and ES cells from the relevant background.

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

1) All mice are monitored daily for signs of discomfort.
2) There are no negative environmental effects; all mice are housed in controlled environments in individually ventilated cages (IVC). Mice get additional cage enrichment (for instance a mouse home and paper tissues) when housed solitarily (while minimized as much as possible, this is sometimes required during the genotype screening phase) and also when signs of distress are observed. In these cases monitoring will be intensified.

Repetition and duplication

E. Repetition

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

n.a.

Accommodation and care

F. Accommodation and care

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

x No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and

provide specific justifications for these choices.

G. Location where the animals procedures are performed

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

x No > Continue with question H.

☐ Yes > Describe this establishment.

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

Classification of discomfort / humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

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

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

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

Anaesthesia according to SOP will be applied when animals are exposed to

-total body and targeted, local irradiation

Anaesthesia and analgesia will be applied according SOP

-during and after surgical removal of small piece of the dorsal skin

-mammary fat pad clearance

-hepatectomy

Analgesia according to SOP will be applied

-after application of tissue damaging agents

I. Other aspects compromising the welfare of the animals

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

Depending on the damaging treatment mice might suffer from anaemia, local pain and general malaise.

Explain why these effects may emerge.

Irradiation causes bone marrow failure leading to anaemia and malaise; other tissue damaging treatments might cause pain due to local inflammatory lesions.

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

The mice are monitored daily. Action will be taken on the same day if unexpected adverse effects show up. In these cases mice will be taken out of the experiment when discomfort exceeds the level of moderate.

J. Humane endpoints

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

☐ No > Continue with question K.

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

In case mice are assessed as having a discomfort level higher than moderate they will not be continued and culled.
We expect a significant number of animals will be taken out of the experiment because of the risk that they will suffer discomfort more than moderate. We are experienced in recognizing the symptoms that are involved in the challenging procedures and therefore we will be able to adequately monitoring these animals, enabling us to prevent suffering of discomfort more than moderate.
We will adhere to the Code of Practice of lab animals used in oncology, in line with internationally agreed rules (see P. Workman et al. Guidelines for welfare and use of animals in cancer research. Br J Cancer 2010; 102: 1555-1577).

In our experiments the most important endpoints that apply are:
-A rapid weight loss of more than 20% of the initial body weight, in case of adult animals. In case of juvenile animals, tailored rules will apply.
-Any sign of tumor formation
-Superficial measurable lesions (by caliper) and/or skin ulceration/necrosis.
-Any abnormal breathing or sign of circulatory problems.
-Any abnormal behavior or locomotion.
-In case of described experimental procedures: any clinical symptoms of discomfort exceeding the level of moderate discomfort to be expected due to the experimental procedures. Any sign of non-healing wounds due to experimental procedures.

Indicate the likely incidence.

The likely incidence of exceeding discomfort level of moderate depends on the treatment. For this incidence we estimate the following for the treatments we will use:

total body irradiation:	<50%
exposure to damaging agents:	<5%
hepatectomy	<20%
other treatments	<5%

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').

For all experimental mice we expect a discomfort level of moderate. The mice are monitored daily. Animals will be taken out of the experiment when discomfort exceeds the level of moderate.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

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

The condition of the animals at the end of the experiment will require that the animal is humanely killed.

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.

x Yes

Centrale Commissie Dierproeven

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> Retouradres Postbus 20401 2500 EK Den Haag

Stichting Het Nederlands Kanker Instituut - Antoni van Leeuwenhoek ziekenhuis
[redacted]
Postbus 90203
1006 BE AMSTERDAM

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

Onze referentie
Aanvraagnummer
AVD301002017840

Bijlagen
1

Datum 27 februari 2017
Betreft Beslissing aanvraag projectvergunning Dierproeven

Geachte [redacted]

Op 20 januari 2017 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Functional analysis of genes implicated in cancer" met aanvraagnummer AVD301002017840. Wij hebben uw aanvraag beoordeeld.

Op 14 februari 2017 heeft u uw aanvraag aangevuld. Op ons verzoek heeft u vraag L bij de verschillende bijlagen ingevuld en heeft u aangegeven gebruik te maken van beide geslachten in bijlagen 3.4.4.2, 3.4.4.3 en 3.4.4.4, tenzij u specifiek relevante redenen heeft om dieren van één geslacht in te zetten in specifieke experimenten. Daarnaast heeft u de humane eindpunten verhelderd.

Beslissing
Wij keuren uw aanvraag goed op grond van artikel 10a van de Wet op de Dierproeven (hierna: de wet). Hierbij gelden de voorwaarden zoals genoemd in de vergunning.
De algemene voorwaarde(n) zijn opgenomen op grond van artikel 1d lid 4, artikel 10a1 lid 2, artikel 10 lid 2 en/of artikel 10a3 van de wet.

U kunt met uw project "Functional analysis of genes implicated in cancer" starten. De vergunning wordt afgegeven van 27 februari 2017 tot en met 1 februari 2022.

Overige wettelijke bepalingen blijven van kracht.

Datum:
27 februari 2017
Aanvraagnummer:
AVD301002017840

Procedure
Bij uw aanvraag heeft u een advies van de Dierexperimentencommissie NKI gevoegd. Dit advies is opgesteld op 20 januari 2017. Bij de beoordeling van uw aanvraag is dit advies betrokken overeenkomstig artikel 10a, lid 3 van de wet. Wij hebben de DEC om aanvullende informatie gevraagd. Op 14 februari 2017 heeft de DEC gereageerd op onze vragen. De DEC heeft aangegeven dat de voorwaarde van beoordeling achteraf ten onrechte in het DEC advies was gesteld en heeft een aangepast advies gestuurd.
Wij kunnen ons vinden in de inhoud van het advies van de Dierexperimentencommissie. Dit advies van de commissie nemen wij over, inclusief de daaraan ten grondslag liggende motivering. Er worden aanvullende algemene voorwaarde(n) gesteld.
Het DEC-advies en de in de bijlage opgenomen beschrijving van de artikelen van de wet- en regelgeving zijn de grondslag van dit besluit.

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

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

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

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

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

Datum:
27 februari 2017
Aanvraagnummer:
AVD301002017840

Centrale Commissie Dierproeven
namens deze:

[redacted]

ir. G. de Peuter
Algemeen Secretaris

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

Centrale Commissie Dierproeven

Projectvergunning

gelet op artikel 10a van de Wet op de Dierproeven

Verleent de Centrale Commissie Dierproeven aan

Naam:	Stichting Het Nederlands Kanker Instituut - Antoni van Leeuwenhoek ziekenhuis
Adres:	Postbus 90203
Postcode en plaats:	1006 BE AMSTERDAM
Deelnemersnummer:	30100

deze projectvergunning voor het tijdvak 27 februari 2017 tot en met 1 februari 2022, voor het project "Functional analysis of genes implicated in cancer" met aanvraagnummer AVD301002017840, volgens advies van Dierexperimentencommissie NKI. Er worden aanvullende algemene voorwaarde(n) gesteld. De functie van de verantwoordelijk onderzoeker is onderzoeker. Voor de uitvoering van het project is Instantie voor Dierenwelzijn verantwoordelijk.
De aanvraag omvat de volgende bescheiden:

1 een aanvraagformulier projectvergunning dierproeven, ontvangen op 20 januari 2017
2 de bij het aanvraagformulier behorende bijlagen:
a Projectvoorstel, zoals ontvangen per digitale indiening op 14 februari 2017;
b Niet-technische Samenvatting van het project, zoals ontvangen per digitale indiening op 14 februari 2017;
c Advies van dierexperimentencommissie d.d. 20 januari 2017, ontvangen op 20 januari 2017.
d De aanvullingen op uw aanvraag, ontvangen op 14 februari 2017

Aanvraagnummer:
AVD301002017840

Naam proef	Diersoort/ Stam	Aantal dieren	Ernst	Opmerkingen
3.4.4.1 General welfare assessment of newly generated genetically modified (GM) mice				
	Muizen (Mus musculus) /	2.050	10% Matig 90% Licht	
3.4.4.2 Validation of conditional genetic modification technology				
	Muizen (Mus musculus) /	2.350	0% Matig 100% Licht	
3.4.4.3 Phenotyping of mice carrying germ line or temporal and/or tissue specific genetic modifications				
	Muizen (Mus musculus) /	9.000	1% Matig 99% Licht	
3.4.4.4 Functional analysis of genetic modifications in mice under challenging conditions				
	Muizen (Mus musculus) /	2.880	100% Matig	

Voorwaarden

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

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

Aanvraagnummer:
AVD301002017840

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

Centrale Commissie Dierproeven

Aanvraagnummer:
AVD301002017840

Weergave wet- en regelgeving

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

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

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

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

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