		Inventaris Wob-verzoek W17-08								
			wordt verstrekt				**weigeringsgronden**			
nr.		**document NTS 2017876**	**reeds openbaar**	**niet**	**geheel**	**deels**	**10.1.c**	**10.2.e**	**10.2.g**	**11.1**
1		Origineel aanvraagformulier				x		x	x	
2		NTS	x							
3		Projectvoorstel				x		x	x	
4		Bijlage 1			x					
5		Bijlage 2			x					
6		Bijlage 3			x					
7		DEC advies				x		x	x	
8		Ontvangstbevestiging				x		x	x	
9		Advies CCD		x						x
10		Beschikking en vergunning				x		x	x	

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Centrale Commissie Dierproeven

1.

23 FEB. 2017

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	1066 CX	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	[redacted]	
Afdeling	[redacted]	
Telefoonnummer	[redacted]	
E-mailadres	[redacted]	

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

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

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

(Titel) Naam en voorletters	[redacted]	☐ Dhr. ☒ Mw.
Functie	[redacted]	
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 - 3 - 2017
	Einddatum	1 - 3 - 2022
3.2 Wat is de titel van het project?	Screening for drivers of gastric cancer	
3.3 Wat is de titel van de niet-technische samenvatting?	Screening voor drijvers van menselijke 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 € 1.541,- 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	[redacted]
Plaats	Amsterdam
Datum	20 - 2 - 2017
Handtekening	[redacted]

Centrale Commissie Dierproeven

3.

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.	Screening for drivers of gastric 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 a major cause of death worldwide. In the Netherlands alone, 130,836 new cases were diagnosed and 43,214 deaths resulted from cancer in 2014 (http://www.cijfersoverkanker.nl/). This translates to a significant burden on patients and their families in terms of suffering and also on society

in a socio-economic context. Therefore, there is a great need for more effective cancer screening and treatments.

Gastric cancer is the 4th most common cancer and the 2nd leading cause of cancer death worldwide [Jemal et. al., 2011]. The 5-year survival rate is <25%, which is due partly to the fact that it is difficult to detect dangerous lesions at an early stage [Marano et. al., 2016]. Additionally, screening for gastric cancer is not routinely performed and, unless a patient has a previously known risk factor, he is not likely to undergo endoscopy or radiography to detect stomach cancer. Finally, there are multiple histological and molecular subtypes, which likely respond to treatment differently.

Approximately 80% of gastric cancer patients inheriting a mutant version of the CDH1 gene, which encodes a protein called E-cadherin, will develop a specific type of gastric cancer called Hereditary Diffuse Gastric Cancer (HDGC). This is an inherited cancer syndrome that leads to an increased risk for both diffuse gastric cancer and lobular breast cancer [Tan et. al., 2015]. Patients who inherit a mutant copy of CDH1 are at high risk for developing gastric cancer at a young age, with an average age at diagnosis of 38 years [Fitzgerald et. al., 2010; Guilford et. al., 1998]. Additionally, female carriers are at an increased risk (50% lifetime risk) for lobular breast cancer [Dossus et. al., 2015]. Currently the only effective treatment is to perform a debilitating surgery to remove the stomach. However, proper timing of the operation is difficult given the absence of a thorough understanding of the multiple steps in gastric cancer carcinogenesis. This prohibits the development of predictive tests, resulting in many patients being either over or under treated.

E-cadherin plays a central role in cell adhesion, allowing cells to stick to one another, and HDGC families are at an increased risk of developing diffuse-type, signet ring cell gastric adenocarcinoma [Guilford et. al., 2010]. Signet ring cells exist as isolated cells, or in small clusters, in the lining of the stomach. Accordingly, diffuse gastric cancer is difficult to diagnose even with routine screening because the cancer often cannot be seen during upper endoscopy. Therefore, most cases of diffuse gastric cancer are diagnosed at late stages (III or IV), when the cancer is incurable.

Although CDH1 mutations confer a high risk of gastric cancer, several studies using mouse models suggest that a CDH1 mutation alone is not sufficient for the development of gastric cancer. In one study, which used a parietal cell-specific E-cadherin knockout mouse, clusters of signet ring-like cells were found, but no invasive gastric adenocarcinomas were observed, supporting the idea that other factors are needed for the development of gastric cancer in a CDH1-mutated background [Mimata et al., 2011]. Furthermore, in a second study, $CDH1^{+/-}$ mice did not develop gastric cancer unless they were treated with the stomach carcinogen, N-methyl-N-nitrosourea [Humar et. al., 2009]. In this study, the authors successfully induced signet-ring cell carcinomas in $CDH1^{+/-}$ mice. However, they did not perform experiments to determine what additional mutations were needed in order to drive tumorigenesis [Humar et al., 2009]. Most recently, the first genetically engineered mouse model of diffuse gastric cancer was created using conditional knockout of both CDH1 and TP53 [Shimada et al., 2012]. Beginning around 6 months of age, these mice developed both intramucosal and invasive cancers, containing poorly differentiated carcinoma and signet-ring cells. By 12 months, all mice had reached humane endpoints due to metastatic gastric cancer. Mice heterozygous for CDH1 and null for TP53, and vice versa, did not develop gastric cancer in this time frame, indicating that deletion of both CDH1 and TP53 is necessary to drive gastric cancer in this setting [Shimada et. al., 2012]. Therefore, it appears that only a single additional mutation, in this particular case in the TP53 gene, is enough to initiate the progression from normal stomach epithelium to stomach cancer in a CDH1 mutated background [Shimada et al., 2012].

Additionally, studies involving the APC gene further support the argument that a single mutation, in addition to CDH1, is enough to drive diffuse gastric cancer within 6-9 months. In a constitutive knockout model, mice heterozygous for both APC and CDH1 develop stomach cancer at 100% penetrance, while mice heterozygous for CDH1 do not develop stomach cancer [Smits et. al. 2000]. Additionally, mice heterozygous for APC developed a single tumor in 4 out of 11 stomachs examined, while mice heterozygous for both APC and CDH1 had at least 1 tumor (range 1-5) in all 14 stomachs examined [Smits et. al., 2000].

The long latency observed in both of the studies mentioned, regardless of mechanism, is ideal for our

purposes, as this gives the patient more time between occurrence of a driver mutation and development of gastric cancer. This allows time for screening (which may be only once per year), analysis of the biopsy, and scheduling surgery.

Since only a single mutation in addition to CDH1 is needed to initiate gastric cancer, it is possible to use an unbiased screening approach to identify additional drivers in a CDH1-mutant background. This notion is supported by a recent publication in non-small cell lung cancer [NSCLC; Chen et al., 2015]. In this paper, the authors use a CRISPR/Cas9 genome-wide knockout library to screen, in vivo, for genes which drive metastasis in non-small cell lung cancer. Starting with a parental NSCLC cell line, containing a mutated TP53 gene and heterozygously mutated KRAS and DICER genes, the authors made a clonal sub-line expressing Cas9-EGFP. Then, they transduced the mouse GeCKOa lentiviral guide RNA library into these cells at a multiplicity of infection (MOI) of 0.4 to generate a pool of mutant cells. After selection, 3x10^7 cells were injected into the right flank of four immunocompromised mice. One mouse was analyzed after 2 weeks to represent an early tumor time point, and three mice were analyzed at 6 weeks to represent a late tumor and, hopefully, metastasis time point. Interestingly, when the mice were analyzed by CT scan, lung metastases were identified in mice injected with the transduced cell pool. When the number of unique gRNAs in the plasmid library, in the transduced cell pools, early and late primary tumors, and lung metastases were quantified, the number of unique gRNAs drops off throughout the evolution of the tumors, indicating enrichment for certain gRNAs over others. Furthermore, in each lobe of the lung, the reads were dominated by one or two gRNAs. This suggests that the metastases were seeded by a small subset of cells from the primary subcutaneous tumor. Hits which accelerate metastasis comprised both well-known tumor suppressors (Nf2, Pten and Cdkn2a) and less well-characterized genes (Trim72, Fga, mir-152 and mir-345). Importantly, the authors investigated whether mutations affecting these genes are found in patients using cBioPortal. Indeed, 75% of the genes targeted by gRNAs enriched in the lung metastases are downregulated in metastatic tumors in NSCLC patients.

Therefore, we are confident that performing a screen, similar to that described above, for genes that drive tumorigenesis will provide important information to achieve our goals. Analogous to the approach used in NSCLC, we will use a CRISPR/Cas9 mediated in vivo approach to identify the genes responsible for progression from normal gastric tissue to gastric cancer.

Once candidate genes are identified, we would like to validate them in an independent study. In vitro and in vivo cancer models have been instrumental in the understanding of the genetics of cancer in order to develop new therapies. The observation that cell lines grow in 2D and can only be established with low efficiency suggests that they do not represent the full spectrum of cancer phenotypes. In an attempt to provide a more physiological model system of primary tumor and normal cells, the organoid platform has been established. Organoids are three-dimensional cell cultures, grown with extracellular matrix-like support and under conditions that mimic the stem cell niche of the relevant tissue. This has resulted in the possibility to culture primary cells of the stomach, gut, liver, pancreas, prostate, and lung with unprecedented success [Boj et. al., 2015; Gao et. al., 2014; Karthaus et. al., 2014; Sachs N. unpublished data]. For example, organoids were established with a 90% success rate from treatment-naive surgical resections of primary colorectal cancers [van de Wetering et. al., 2015] and as high as 71% from needle biopsies of metastases [Weeber et. al., 2015]. Importantly, organoids maintain the mutational make-up of the original tumor tissue [Weeber et. al., 2015]. This provides a unique opportunity to study cancer in a personalized manner. Moreover, healthy and tumor-derived organoid cultures can be genetically manipulated allowing for functional analysis of individual mutations in both tumor and normal cells. Therefore, tumor organoids have the potential to revolutionize cancer medicine by providing an ex vivo test platform for the individual patient. We aim to utilize organoids, combined with mouse models of gastric cancer, to validate hits from our in vivo screen for drivers of CDH1-mutated gastric cancer to improve screening in HDGC families.

In this way, we aim to dissect the genetics of gastric tumorigenesis in order to develop a test that will improve surveillance in HDGC families and allow timely surgery. For a visual overview of the flow of our project application, please see the attached flowchart.

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?

Research objective:

Our overarching goal is to use organoids, in combination with mouse models of gastric cancer, to validate hits from our in vivo screen for drivers of CDH1-mutated gastric cancer to improve screening in HDGC families.

The key factor in this goal is to identify genes that drive the progression from normal gastric epithelial tissue to cancer. As such, we aim to:

1. Identify candidate genes involved in HDGC.
2. Validate those candidate genes.
3. Explore the role of validated candidate genes in malignancy.

Achievability:

We believe that this objective is achievable for several reasons:

1. Previous studies indicate that only one mutation in addition to CDH1, for example either TP53 or APC, is sufficient to drive gastric tumorigenesis [Shimada et al., 2012; Humar et al., 2009; Smits et. al., 2000]. This provides confidence that our screen, starting with a CDH1 mutated genetic background, will result in additional hits that drive gastric cancer.
2. A similar CRISPR in vivo screening approach has been used in NSCLC to successfully identify genes involved in metastasis [Chen et al., 2015].
3. Two independent groups have successfully used targeted mutations made by the CRISPR/Cas9 method in colorectal organoids to identify the genes which drive tumorigenesis in this tissue [Matano et. al., 2015; Drost et. al., 2015].
4. All of the technologies that we will use in this project are established and published. Additionally, we have existing collaborations with several of the labs which have developed these technologies to help ensure the success of this project in our hands.

3.3 Relevance

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

Scientific relevance:

We hope that our contributions will be two-fold. First, we aim to shed light on the genetic mutations that are required, in combination with a CDH1 mutation, to drive the progression from normal stomach tissue to gastric cancer. Although we focus on CDH1 mutated gastric cancer in this proposal, it is also possible that any driver mutations we identify may be important in CDH1 wildtype gastric cancer as well. As we plan to do these experiments in both human and mouse organoids, we will also explore the similarities and differences in the genes responsible for progression of human and mouse gastric cancer. Second, we expect these insights will lead to changes in patient screening, which will allow us to develop a genetic test to identify potentially harmful cells at an earlier time point.

Social relevance:

Individuals who are known to carry a CDH1 mutation are at high risk of developing one of the most difficult to treat cancers: gastric cancer. To live life in constant fear of developing cancer at a young age is a huge burden, not only for the affected individual, but also for their families. The only potential cure is to surgically remove the stomach at an early age. This has significant impact on patient quality of life. Developing a test that can predict whether there is, indeed, the need for surgery may reduce this burden for the families and give them a better perspective. In this project, we aim to improve screening for gastric cancer in families at risk for developing hereditary diffuse gastric cancer, in order to decrease cancer-associated death and to improve patient quality of life.

3.4 Research strategy

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

To identify the genes that will be included in our screen, we have used cBioPortal and filtered for all gastric cancers containing a CDH1 mutation. Within this subset of patients, we looked for all genes that

are mutated at any percentage, which resulted in a list of 865 genes. We then extracted gRNAs for these genes from existing, validated whole genome mouse CRISPR libraries. These gRNAs, as well as 200 non-targeting gRNAs, will be cloned into a lentiviral gRNA expression vector and the cloned library will be sequenced to ensure that complexity is maintained. This lentiviral gRNA library will then be used to perform our in vivo screen in mice.

As the immune system and tumorigenesis are intimately linked, we have elected to perform this screen using immune competent mice containing CDH1 and Cas9 conditional alleles. Importantly, these mice are known to be immune tolerant to Cas9 expression [Annunziato et al., 2016]. To perform the screen, we will delete CDH1 and activate Cas9 specifically in the stomach using a lentivirally delivered Cre. We have chosen this approach because the cells from which diffuse gastric cancer originates have not yet been identified. By choosing this method of Cre delivery, we are able to delete CDH1 and activate Cas9 in all cell types in the stomach epithelium.

The lentiviral gRNA library itself will be cloned into the same plasmid and, therefore, delivered in the same manner and at the same time as the lentiviral Cre. We will do this using a commercially available kit designed for in vivo use and which has been shown to work successfully for lentiviral transduction of the stomach epithelium, and which also indicates that lentiviral transduction in the acid environment of the stomach is possible [Ozbiosciences; Scherer et. al., 2002]. Briefly, the lentiviral particles are mixed with magnetic particles and injected into the stomach. A magnet is placed under the stomach for 20 minutes to allow transduction of the stomach epithelium to occur. Using previous studies as a guide, the mice will be sacrificed at four time points, the stomachs removed and submitted for pathological examination [Shimada et. al., 2012; Humar et. al., 2009].

After we have identified a candidate list of genes from our in vivo screen, we will select 10-15 of our top candidates for validation using an orthotopic mouse model. We will do this based on the significance of enrichment of each gRNA, the frequency which these mutations are found in CDH1 mutated gastric cancer patients, and the function of each candidate. Then, we will use the CRISPR/Cas9 system to engineer CDH1-mutated, but non-cancerous, gastric organoid lines to contain mutations in our candidate genes. We will do this to test the ability of each line to form tumors in an orthotopic mouse model of gastric cancer. To confirm that the hits we identify in mice translate to the patient setting, we will validate our hits with both mouse and human CDH1 mutant gastric organoids.

We would additionally like to identify combinations of mutations which are likely to result in more aggressive gastric carcinomas. We will do this by creating an allelic series ranging from single, to all possible combinations of double, and triple mutations in genes commonly co-mutated with CDH1 and which are identified as hits in our screen. We will also perform this experiment with three genes previously identified as drivers of gastric cancer by the TCGA and which also co-occur with CDH1 mutations [Wang et. al., 2014; cBioPortal].

The engineered organoids will then be orthotopically implanted and allowed to grow for up to 12 months. Although several orthotopic models of gastric cancer are available, we have elected to use the published, non-surgical, electrocoagulation-based orthotopic mouse model of gastric cancer, as this model most closely mimics the development of gastric cancer in the human setting and will cause the least discomfort out of the available orthotopic methods [Bhullar et al., 2010]. The technology for this method is in place in collaboration with the group in the United States that developed the method. At multiple time points within the 12 month time frame, mice will be sacrificed and submitted to the NKI Animal Pathology Department for pathological evaluation of gastric tumor growth. This will provide us information on the critical limit regarding the number of mutations needed in order for normal gastric tissue to transition to gastric cancer in a CDH1 mutated background. It will also provide insight on whether the specific mutations, rather than the sheer number of mutations, is important. We additionally hope to correlate our data with malignancy.

It worth mentioning that it is important to validate the results of our screen in an orthotopic setting rather than a subcutaneous setting, for 2 reasons: 1.) orthotopic models provide several important advantages over other systems, including physiologically relevant:

progression of disease, tumor-host interactions, development of metastases, and organ-specific gene expression [Killion et. al., 1998] 2.) we aim to identify early drivers of gastric cancer, thus we expect the resulting lesions to be very small and minimally invasive and gastric cancer almost never metastasizes to the subcutaneous space suggesting that this environment is not supportive enough for minimally invasive gastric tumor growth. Therefore, it is highly likely that our engineered organoids would not grow out in the subcutaneous setting and we would miss out on validating important hits. For this same reason, it is not possible to monitor our mice by in vivo imaging techniques, as we expect the lesions to be smaller than the limit of detection.

Later in the application, we propose to test a subset of organoid lines in a subcutaneous model. However, this subset will be composed of organoid lines that contain combinations of mutations, rather than single driver mutations, and which have already been shown in either the orthotopic model or by in vitro tests to be highly malignant. This platform will not be used to confirm the driver mutations identified by our screen.

We recognize that the orthotopic technique induces more discomfort to the animals and can be more difficult to perform in a reproducible manner. However, as our results will be used to guide patient treatment, it is of the utmost importance that we mimic the physiological setting as closely as possible. In order to make this technique as reproducible as we can, we have requested mice for pilot studies not only to establish and optimize the method in our institute, but also for training animal technicians in performing this technique. We feel that these pilots are important since, with optimized protocols and well-trained technicians, the results of an experiment become more reliable and require less mice.

We would also like to test our more malignant engineered organoid lines in a subcutaneous model. This is an important step, as it will provide us with information regarding whether any of the driver mutations that we identify lead to particularly aggressive gastric cancer. If the tumors grow subcutaneously, this will allow us to both confirm the tumorigenicity of these engineered organoid lines and allow us to look at the invasiveness of the lines. It will also provide us with a tool to grow a larger number of tumor cells for further genetic and biochemical analyses. Plus, it has the added advantage that these tumors, which are likely to be more aggressive, are visible under the skin (rather than internal) and can be closely monitored to minimize animal suffering.

This project has direct clinical relevance and will allow us to provide better screening in CDH1 mutation carriers, resulting in more personalized treatment regimens - to prevent overtreatment of some patients and to provide early, aggressive treatments to those who would benefit. Once we have the information on which mutations are needed to develop gastric cancer, we will develop a genetic test which can be used to screen biopsies obtained from patients in order to identify whether the patient has developed mutations in any of these genes. This will allow us to identify cancerous cells at an early time point, hopefully even before they have developed into a tumor. Since HDGC is difficult to diagnose by current methods, we hope that this will be a step forward in screening these families and provide a tool for a more timely (i.e. delayed) removal of the stomach or, in some cases, not removing the stomach, since penetrance of the CDH1 mutation is not 100%.

For a visual overview of the project proposal, including decision points, please see the included flowchart.

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.

1. **<u>Perform an unbiased screen for drivers of CDH1-mutated diffuse gastric cancer</u>**

We will use mice containing CDH1 and Cas9 conditional alleles from Stefano Annunziato (Jos Jonkers's lab at the Netherlands Cancer Institute), as well as a lentivirally delivered Cre protein, to delete CDH1 and activate Cas9 specifically in the stomach. A lentiviral gRNA library will be cloned into the same plasmid as the Cre and, therefore, will be delivered concurrently. We will do this by surgically opening the abdomen of an anesthetized mouse and delivering the magnetized Cre/gRNA library in a buffer

solution by injection into the stomach. A magnet will be placed under the stomach and, after 20 minutes, the magnet will be removed and the abdomen of the mouse surgically closed [Scherer et. al., 2002]. Mice will also be treated with analgesics before and after surgery for pain management. At 4 time points, the mice will be sacrificed and the stomachs submitted to the NKI Animal Pathology department for sectioning, H&E staining, and pathological examination. Stomach tissue will then be scraped from the slides and submitted for sequencing to identify candidate gastric cancer driver mutations.

2. Validate hits in an orthotopic model of gastric cancer

To validate the hits from the in vivo screen, we will first engineer organoid lines to contain mutations in our genes of interest using the CRISPR/Cas9 system. Then we will follow the procedures of an established orthotopic model of gastric cancer using a published electrocoagulation-based method [Bhullar et. al., 2013]. This involves placing a flexible plastic gavage tube down the throat of an anesthetized mouse. Next, a rounded-tip metal electrode is passed down the gavage tube and a low dose of electricity is applied, causing a superficial injury to the gastric mucosa. The electrode is removed and organoids in a basement membrane mimic are injected down the gavage tube to the site of superficial injury. Based on previous publications (and the results from the in vivo screen) we can expect that gastric tumors will form within 12 months and can then be used for further study (pathological examination, DNA, RNA, protein isolation, etc.) [Shimada et al., 2012; Humar et al., 2009]. We will first troubleshoot using human gastric cancer organoids from our in-lab biobank and mouse cancer cells, then we will use this system to test the tumorigenic potential of each of the mutant human and mouse organoid lines *in vivo.*

3. Test for invasiveness in a subcutaneous model of gastric cancer

We would also like to explore the malignancy of a subset of our engineered organoid lines in a subcutaneous model of gastric cancer. This will allow us to examine the invasiveness of the lines and it will provide us with a tool to grow a larger number of tumor cells for further genetic and biochemical analyses. This method involves subcutaneous injection of our organoids of interest in the rear flank of an anaesthetized mouse, followed by a waiting and monitoring period of up to 12 months (or until the resulting tumor reaches a volume of 1500mm^3, whichever occurs first).

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.

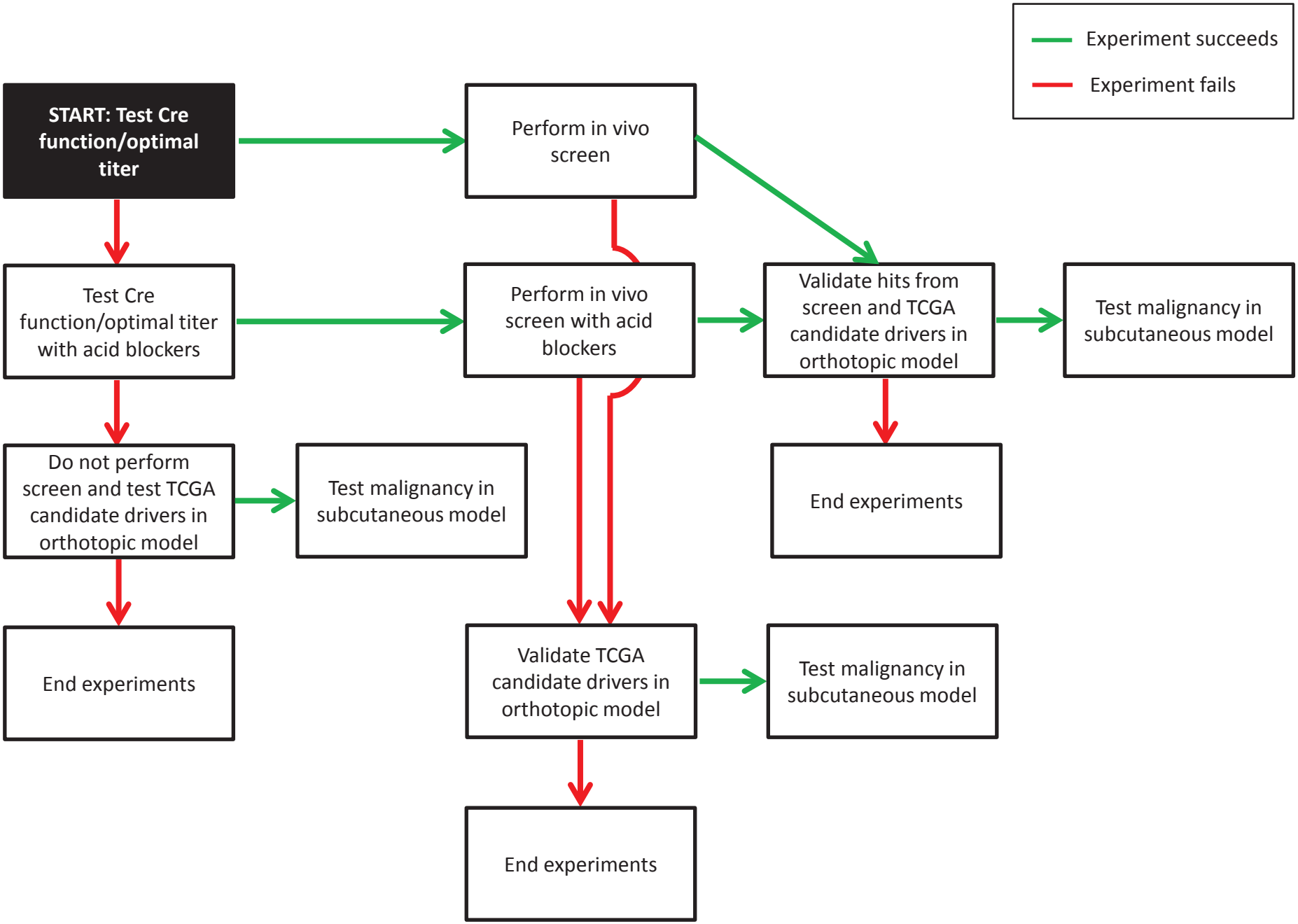
This project application is based on the identification and validation of genes which lead to the initiation of diffuse gastric cancer in a CDH1 mutated genetic background. The experiments described in this project proposal are all focused on the identification and validation of these genes, which will allow us to develop a genetic test to be used in the clinic to identify early signs of progression of gastric cancer. Therefore, the overall aim of this project is twofold: 1.) to better understand the genetics behind hereditary gastric cancer, which will allow us to 2.) explore better screening options that may be applied to the clinic. The major milestones of this project are:

1. Perform an unbiased, in vivo screen for drivers of CDH1-mutated diffuse gastric cancer.
2. Validate hits in an orthotopic model of gastric cancer.
3. Test for invasiveness in a subcutaneous model of gastric cancer.

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	In vivo screen for drivers of gastric cancer
2	Orthotopic models of gastric cancer
3	Subcutaneous models of gastric cancer
4	
5	
6	
7	

8	
9	
10	


Experiment succeeds
Experiment fails
START: Test Cre function/optimal titer
Perform in vivo screen
Test Cre function/optimal titer with acid blockers
Perform in vivo screen with acid blockers
Validate hits from screen and TCGA candidate drivers in orthotopic model
Test malignancy in subcutaneous model
Do not perform screen and test TCGA candidate drivers in orthotopic model
Test malignancy in subcutaneous model
End experiments
End experiments
Validate TCGA candidate drivers in orthotopic model
Test malignancy in subcutaneous model
End experiments

Centrale Commissie Dierproeven

4.

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.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	In vivo screen for drivers of gastric cancer

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.

This appendix describes an unbiased in vivo screen to identify drivers of gastric cancer in a CDH1 mutated background.

The screen will be performed in immune competent mice containing CDH1 and Cas9 conditional alleles (from Jos Jonkers's lab at the Netherlands Cancer Institute). Importantly, these mice are known to be immune tolerant to Cas9 expression [Annunziato et al., 2016]. We will use a lentivirally delivered Cre protein to delete CDH1 and activate Cas9 specifically in the stomach, in combination with a lentiviral gRNA library, which has been cloned into the same plasmid as the lentiviral Cre (pLentiCre). In vitro tests with organoids containing a conditional CDH1 allele and electroporated with pLentiCre have confirmed that the Cre cassette is functional. We have chosen to use a lentivirally delivered Cre, rather than a traditional promoter-driven Cre, because it is not yet known which cells in the stomach epithelium are the origin of diffuse gastric cancer. We will perform the screen by combining the Cre/gRNA library mixture with commercially available magnetic particles, which are designed for in vivo use and which have been used successfully by other groups for lentiviral transduction of the stomach epithelium, indicating that transduction in the acid environment of the stomach is possible [Magnetofection, Ozbiosciences; Scherer et. al., 2002]. Then, we will surgically open the abdomen of an anesthetized mouse and deliver 0.2mL of the Cre/gRNA library mixture in a buffer solution by injection into the stomach. A commercially available magnet will be placed under the stomach and, after 20 minutes, the magnet will be removed and the abdomen of the mouse surgically closed [protocol, Ozbiosciences]. Since stomach emptying time is, on average 2 minutes (+/- 1 minute) in fasted mice, the magnet ensures that the viral particles are kept in contact with the stomach epithelium long enough for viral

transduction to occur [Roda et. al., 2010]. We have elected to use this method, rather than a gastric clamp to prevent stomach emptying during the 20 minute treatment, because it will avoid the possible risk of additional discomfort due to ischemia. Mice will also be treated with analgesics before and after surgery for pain management. At multiple time points post-treatment, the mice will be sacrificed and the stomachs submitted to the NKI Animal Pathology department for sectioning, H&E staining, and pathological examination. After scanning the slide images into a digital image storage program, areas affected by gastric cancer will be identified by a pathologist. These areas will then be manually scraped from the slides using a razor blade. DNA will be extracted from the scraped tissue and submitted for sequencing to identify candidate gastric cancer driver mutations.

In order to perform our screen using the correct conditions, which will not only ensure reliable results but will also minimize the number of mice needed and the discomfort caused to each mouse, we will need to perform some optimization experiments. First, we will need to validate the in vivo viral transduction method and ensure that our lentivirally delivered Cre is catalytically active in the acid environment of the stomach. We will also perform an experiment to determine the optimal lentiviral titer to use for in vivo transduction. Only after we have ensured that the Cre is functional, and we have identified the optimal lentiviral titer to use for in vivo transduction, will we proceed with the screen.

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

The aim of this procedure is to allow us to identify drivers of CDH1-mutated gastric cancer. We will do this using a lentivirally delivered Cre on the same plasmid as a gRNA library targeting all genes known to be mutated in CDH1-mutated gastric cancer in humans.

Nature of procedure: This procedure involves fasting the mice overnight (with free access to water) before the procedure to ensure an empty stomach in preparation for treatment with lentiviral particles the next day. Pain relief will be administered prior to surgery and post-surgery according to the NKI "Anaesthesia and Pain Relief SOP". Anaesthesia will be given by isoflurane, according to the NKI "Anaesthesia and Pain Relief SOP", then we will surgically open the abdomen of an anesthetized mouse and deliver 0.2mL of magnetized Cre/gRNA library lentivirus in a buffer solution by injection into the stomach. A commercially available magnet will be placed under the stomach and, after 20 minutes, the magnet will be removed and the abdomen of the mouse surgically closed. Mice will be treated with analgesics before and after surgery for pain management. The mouse will be kept warm during the procedure using a heating pad and body temperature will be monitored to prevent under- or overheating.

First, we will need to perform up to 3 validation experiments: 1.) to ensure that the in vivo viral transduction method works 2.) to determine whether our Cre construct results in effective Cre activity and 3.) to determine whether acid blockers enhance in vivo viral transduction. To test point 1.) and point 2.), we will perform an experiment to determine the optimal lentiviral titer to use for in vivo transduction. We will do this using mT/mG reporter mice and lentivirally delivered Cre, which will give a visual readout of transduction efficiency: if cells are green, they have received a catalytically active Cre protein; if cells are red, they have not. Since we want to perform our screen at a low multiplicity of infection, we will choose a lentiviral titer that results in not more than 50% of the cells expressing GFP. Of course, it is possible that the acidic environment of the stomach will interfere with the efficiency of lentiviral transduction. If we do not see evidence of GFP expression at any of the viral concentrations that we test, we will address point 3.) and repeat the experiment with the additional treatment of acid blockers. If treatment with acid blockers does not increase the transduction efficiency, we will not use them in the actual in-vivo screen described below, since it causes additional discomfort to the mice with no additional benefit. Alternatively, if we do not see evidence of GFP expression at any of the viral concentrations that we test, and with the inclusion of acid blockers, then we will not proceed with the screen. Once we have identified the optimal lentiviral titer to use for in vivo transduction, and whether acid blockers are needed, we will proceed with the screen.

Within 12 months, the mice will be sacrificed and the stomachs submitted to the NKI Animal Pathology department for sectioning, H&E staining, and pathological examination. After scanning the slide images into a digital image storage program, the stomach tissue will be scraped from the slides and submitted

for sequencing to identify candidate gastric cancer driver mutations.

Frequency and duration of treatment: Each mouse will be subjected to this procedure one time and we expect the procedure to last approximately 45 minutes.

Justification for the selected approach: We have chosen this approach because it will allow us to perform an unbiased screen for drivers of CDH1-mutated gastric cancer. By including gRNAs against all genes known to be mutated in CDH1-mutated gastric cancer in humans, we feel we have the best chance of identifying those genes which drive gastric cancer in a CDH1-mutated background, while using the smallest number of animals possible.

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

For the initial set-up experiment, we would like to request 20 animals per group. Since this method has not been performed previously in the institute, we expect this first experiment to require more animals than we would normally request. For all of the following set-up and optimization experiments, we would like to request 10 animals per group. This number is based on institute-wide experience with setting up mouse models of cancer.

For our in vivo screen, we would like to request 5 animals per control group and 10 animals per experimental group. As a supporting calculation we used a One Sided T-test. In this calculation, we assume a mean of 1 for the control group (number of significantly enriched gRNAs) and 10 for the experimental groups, and a variance of 1 and 5, respectively.

B. The animals

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

Species: mus musculus.

Origin: mT/mG mice (own breeding or commercial supplier) and mice containing conditional alleles for CDH1 and Cas9 (own breeding).

Life stage: adult (both male and female, in proportional quantities).

Justification: Mice are a commonly used animal model in oncology studies due to their short generation time and ease of genetic manipulation. Mice also share a similar organ structure to humans and have considerable genetic conservation as compared to humans. Furthermore, much research using mice has been conducted, resulting in the availability of many genome-wide data sets and advanced tools for genetic and biological manipulation, including tumor models, cell lines, and xenograft platforms.

Estimated numbers for in-vivo screen:
In vivo screen set-up pilot:
As we would like to transduce the cells of the stomach epithelium at a low multiplicity of infection, we will first need to experimentally determine the optimal viral titre to use. We can do this with a lentiviral Cre protein and mT/mG mice, which expresses membrane-targeted tandem dimer Tomato (mT) prior to Cre-mediated excision and membrane-targeted green fluorescent protein (mG) after excision [Muzumdar et. al. 2007]. Therefore, cells which receive a catalytically active viral Cre protein will become green. We will aim for a viral titre which results in GFP expression in the greatest percentage, but not more than 50%, of the stomach epithelial cells. To determine the percentage of stomach cells which have received an active lentiviral Cre, we will sacrifice the mice after 4 days in order to remove the stomachs for molecular examination.

Based on published experiments by other groups, we would like to test a range of lentiviral titres from 10^3 to 10^11 PFU per mL [Afrazi et. al., 2014; Polyak et. al., 2008; Scherer et. al., 2002].
Since all new procedures have a learning curve, and we need to establish this method in our institute, we would like to use 20 animals per group in 6 groups, covering a range of viral titers:

1. Buffer without lentiviral Cre
2. Lentiviral Cre in buffer at viral titre #1
3. Lentiviral Cre in buffer at viral titre #2
4. Lentiviral Cre in buffer at viral titre #3
5. Lentiviral Cre in buffer at viral titre #4
6. Lentiviral Cre in buffer at viral titre #5

Therefore, we will use 120 mice for this pilot study.

If we find that the cells of the stomach are being transduced at a very low efficiency, it is possible that this is due to the acidic environment of the stomach dissolving the lentiviral particles or inactivating the Cre protein. Therefore, in this case, we will repeat this set-up pilot in mT/mG mice with the addition that we will also treat the mice with acid blockers. We will follow a previously published protocol, whereby mice receive subcutaneous pantoprazole (0.4 mg in 0.2 mL) twice daily for 2 days prior to the lentiviral infusion [Stiefel et. al., 2006]. Then we will follow the same procedure as above.

Again, based on published experiments by other groups, we would like to test a range of lentiviral titres from 10^3 to 10^11 PFU per mL [Afrazi et. al., 2014; Polyak et. al., 2008; Scherer et. al., 2002]. In this pilot study, we would like to use 10 animals per group in 6 groups, covering a range of viral titers:
1. Buffer without lentiviral Cre
2. Lentiviral Cre in buffer at viral titre #1
3. Lentiviral Cre in buffer at viral titre #2
4. Lentiviral Cre in buffer at viral titre #3
5. Lentiviral Cre in buffer at viral titre #4
6. Lentiviral Cre in buffer at viral titre #5

Therefore, we will use 60 mice for this pilot study, if it is necessary. If treatment with acid blockers does not increase the transduction efficiency, we will not use them in the actual in-vivo screen described below, since it causes additional discomfort to the mice with no additional benefit.

In-vivo screen training:
As we may make advantage of the Animal Intervention Unit at the NKI, which is available to perform experiments on behalf of researchers, we would like to train up to a total of 4 staff from the Intervention Unit. We will perform the experiments as described above (with or without acid blockers, as needed, based on the results of the experiment above) and would like to use 10 mT/mG mice per trainee, which we think will allow us sufficient practice in establishing the method. We will perform these training sessions using the lentiviral Cre titer identified in the pilot experiments above. **We feel this training is important since, with well-trained technicians, the results of an experiment become more reliable and require less mice**. Since this is a training step and not an experiment which requires extensive controls, we will use only a single group of mice:

1. Lentiviral Cre in buffer at previously identified viral titre

To determine the percentage of stomach cells which have received an active lentiviral Cre, we will sacrifice the mice after 4 days in order to remove the stomachs for molecular examination.
Please note that we do not plain to train all 4 people at once, but training will be done as needed over a period of 5 years. Therefore, we will use a *maximum* of 40 mice for this training.

In vivo screen experimental set-up:
For the in vivo screen, we will use mice containing a conditional CDH1 and Cas9 allele. If necessary, based on the findings of the acid-blocker experiment described above, mice will receive subcutaneous pantoprazole (0.4 mg in 0.2 mL) twice daily for 2 days prior to the procedure [Stiefel et. al., 2006].

We would like to include 6 time points to ensure that we do not miss any hits – either particularly aggressive hits or hits which result in later development of gastric cancer. Based on previous publications, we can expect a time range between 2-12 months is reasonable [Shimada et al., 2012; Humar et al., 2009]. A typical study will contain 12 control groups (one negative control group at each

time point and one positive control group at each time point), with 5 mice per group:

1. Mice treated with buffer only and sacrificed at post-treatment time point 1
2. Mice treated with buffer only and sacrificed at post-treatment time point 2
3. Mice treated with buffer only and sacrificed at post-treatment time point 3
4. Mice treated with buffer only and sacrificed at post-treatment time point 4
5. Mice treated with buffer only and sacrificed at post-treatment time point 5
6. Mice treated with buffer only and sacrificed at post-treatment time point 6
7. Mice treated with lentiviral Cre and gRNAs against TP53 and sacrificed at post-treatment time point 1
8. Mice treated with lentiviral Cre and gRNAs against TP53 and sacrificed at post-treatment time point 2
9. Mice treated with lentiviral Cre and gRNAs against TP53 and sacrificed at post-treatment time point 3
10. Mice treated with lentiviral Cre and gRNAs against TP53 and sacrificed at post-treatment time point 4
11. Mice treated with lentiviral Cre and gRNAs against TP53 and sacrificed at post-treatment time point 5
12. Mice treated with lentiviral Cre and gRNAs against TP53 and sacrificed at post-treatment time point 6

And 6 experimental groups, with 10 mice per group:

1. Mice treated with lentiviral Cre and the experimental gRNA library and sacrificed at post-treatment time point 1
2. Mice treated with lentiviral Cre and the experimental gRNA library and sacrificed at post-treatment time point 2
3. Mice treated with lentiviral Cre and the experimental gRNA library and sacrificed at post-treatment time point 3
4. Mice treated with lentiviral Cre and the experimental gRNA library and sacrificed at post-treatment time point 4
5. Mice treated with lentiviral Cre and the experimental gRNA library and sacrificed at post-treatment time point 5
6. Mice treated with lentiviral Cre and the experimental gRNA library and sacrificed at post-treatment time point 6

Therefore, one repetition of the *in vivo* screen will require 120 mice. We would like to repeat the experiment three independent times, which results in a total of 360 mice over 5 years.

The total number of mice requested for the in-vivo screen section of the proposal is: 580

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.

Replacement:
In silico research will be performed and critically evaluated prior to beginning any in vivo work. However,

as cancer is a complex disease, and we hope to translate our findings to the clinical setting, our experiments cannot be limited to results from in silico analysis. Organoids, cells, and computer models are not yet sophisticated enough to mimic factors such as the stroma, oxygen supply, the immune system, and metabolism, and therefore cannot yet replace in vivo screens.

Reduction:

We have elected to perform an unbiased screen for drivers of gastric cancer, as this provides us with a balance between having the best chance of identifying those genes which drive gastric cancer in a CDH1-mutated background, while using the smallest number of animals possible. The proposed number of animals per group (n=5-20 animals) is based on institute-wide experience with these types of experiments, as well as statistical calculations. Further reduction of the number of animals per cohort would decrease the statistical power of the experiments.

Refinement:

By performing pilots to set up the method, to optimize the method, and to properly train personnel, we aim to minimize the discomfort to the animals involved. Additionally, we will administer analgesics according to the NKI "Anaesthesia and Pain Relief SOP" and mice will be closely monitored for signs of discomfort. If we have to test the effect of acid blockers and we find that their use confers no experimental advantage, we will not we will not use them in the actual in-vivo screen described below, since the extra treatment will, by definition, cause additional discomfort to the mice. The procedure will be performed only once, under anaesthesia, and in an appropriate volume (0.2mL). We have also chosen this procedure over another available procedure involving a gastric clamp to prevent stomach emptying, which could cause ischemia and result in additional discomfort to the mice. Mice will be closely monitored during the immediate post-surgical period.

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

We realize that the procedures described in this appendix will inevitably cause stress and suffering to the animals. In order to minimize these effects, we will adhere to the general and internationally accepted rules (Code of Practice) of handling lab animals in oncology [Workman et. al., 2010]. Under these rules, the animals will be humanely killed when any humane endpoint is reached. Additionally, mice will be anaesthetized during the surgical procedure and treated with analgesics before and after surgery for pain management according to the NKI "Anaesthesia and Pain Relief SOP". Furthermore, mice are housed in state-of-the-art, individually ventilated cages. As standard procedure, mice in our facility are group-housed and provided with cage enrichment, bedding, and free access to food and water. To minimize adverse effects on the environment, procedures requiring the use of lentivirus will be done in a special ML-II certified sub-facility within our animal facility.

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.

Not applicable.

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.

Within the NKI, we have developed a standard operation protocol that describes the most appropriate methods for anaesthesia and analgesia for each (surgical) procedure. This protocol has been developed by the animal welfare officer of the NKI and may be subject to change when new concepts or ideas about optimal anaesthesia/analgesia evolve. Based on the current protocol, the most appropriate anaesthetic for our application is isofluorane and analgesics are Rimadyl and Temgesic.

I. Other aspects compromising the welfare of the animals

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

Similar to cancer patients, animals carrying tumors in internal organs may develop dysfunction of the involved organs, or other complications due to metastasis (although we do not expect this to occur as we aim to induce minimal mutational burden and looking for initial drivers of tumorigenesis). Although, based on previously published results [Shimada et al., 2012; Humar et al., 2009], we expect that all mice will be sacrificed before a humane endpoint is reached, the mice may undergo moderate discomfort due to tumor growth. Specifically in the case of gastrointestinal tumors, obstruction of the gastro-intestinal tract may occur. Alternatively, the mice may experience weight loss, blood in feces, diarrhea, or obstruction of the GI tract. Mice which undergo surgery may experience improper wound healing or infection due to the surgical procedure. Should any signs of discomfort be detected, the mouse will be observed daily and the decision to sacrifice the mouse will be made with the help of the NKI animal welfare officers. If a humane endpoint is reached, the mouse will be sacrificed immediately

Explain why these effects may emerge.

These effects are an unavoidable consequence of tumor growth.

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

In general, the effects on the wellbeing of the mice due to tumor growth cannot be completely avoided. In order to minimize the burden on the mice, they will be monitored for signs of distress (hunched back, lack of grooming, blood in feces, signs of infection, significant weight loss) and killed when any of the humane endpoints described below are met. Nevertheless, unforeseen complications may arise. In such cases, we will attempt to find solutions which minimize the impact of unforeseen complications, for example, by providing easy access to food (mush-feeding), taking into account the humane endpoints below.

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 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 general, the most important humane endpoints that apply are:

- Lack of wound healing after surgery.
- Signs of infection after surgery.
- Weight loss of more than 20% of the initial body weight, measured from the start of treatment of adult animals.
- Severe abnormal breathing.
- Evidence of abnormal stool (for example, blood in the stool).
- Severe abnormal behavior (for example: lack of grooming, humched posture, pilo-erection).

Indicate the likely incidence.

For the in vivo screen, we expect that all mice will develop gastric cancer. But, since we are infecting at a low MOI and previously identified (i.e. the most aggressive) drivers take 6-9 months for gastric cancer to form, we do not expect any mice to reach a humane endpoint [Shimada et al., 2012; Humar et al., 2009].

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').

Our in vivo screen involves surgery under anaesthesia and the development of tumors in the stomach, an internal organ. Although we expect that all mice will be sacrificed before a humane endpoint is reached, the mice may undergo moderate discomfort due to tumor growth, including weight loss, blood in feces, diarrhea, or obstruction of the GI tract. As all mice will undergo surgery under anaesthesia and are also expected to develop gastric cancer, we expect 100% of our mice to experience moderate discomfort.

Additional discomfort due to procedures:
Simple but frequent handlings, like weighing: mild discomfort.

We expect that mice under this appendix will experience mild, moderate, and severe discomfort in: 0, 100, and 0% of the cases, respectively.

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.

In order to do a full pathology exam on the tumors, and necropsy on the mice (to look for metastases), it is necessary to remove the affected organ(s). Therefore, the mice will need to be sacrificed at the end of the experiment.

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

5.

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.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
2	Orthotopic models of gastric cancer

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.

This appendix describes the validation of candidate drivers of CDH1-mutated gastric cancer using an in vivo orthotopic mouse model.

For this, we will engineer CDH1-mutated, but non-cancerous, gastric organoid lines to contain mutations in candidate genes in order to test the ability of each line to form tumors in an orthotopic mouse model of gastric cancer. We will prioritize candidates based on the significance of enrichment of each gRNA, the frequency which these mutations are found in CDH1 mutated gastric cancer patients, and the function of each candidate gene. In order to confirm that the hits we identify in mice translate to the patient setting, we will validate our hits with both mouse and human CDH1 mutant gastric organoids. Accordingly, we will use both CDH1/Cas9 conditional mouse gastric organoid lines, and CDH1 mutant human gastric organoid lines, both of which we already have growing in the lab, to validate our hits. For this validation test, we will create organoid lines with targeted mutations in these genes using the CRISPR/Cas9 genome editing system. Then we will test the tumorigenic potential of the individual lines in a non-surgical orthotopic mouse model [Bhullar et al., 2010]. The technology for this method is in place in collaboration with the group in the United States that developed the method and we will troubleshoot the system first using organoids/cells that are known to grow well in vitro. If we are unable to optimize the system at this stage, we will not proceed.

It worth mentioning that it is highly likely that our engineered organoids would not grow out in the subcutaneous setting and we would miss out on validating important hits. For this same reason, it is not possible to monitor our mice by in vivo imaging techniques, as we expect the

lesions to be smaller than the limit of detection.

Gastric tumors are expected to form within 12 months and can then be used for further study (pathological examination, DNA, RNA, protein isolation, etc.) [Shimada et. al., 2012; Humar et. al., 2009]. At multiple time points after orthotopic implantation, but within 12 months after the procedure, the mice will be sacrificed and submitted to the NKI Mouse Pathology Department for pathological examination to determine whether tumors have formed.

We would also like to identify combinations of mutations which are likely to result in more aggressive gastric carcinomas. We will do this by creating an allelic series ranging from single, to all possible combinations of double and triple mutations of those genes which are identified as bona fide hits in the previous experiment. To select the genes which will be tested in this experiment, we will take into account the malignancy of the mutations observed in the previous orthotopic validation experiment, as well as the frequency with which the genes are mutated in patient samples. Included in our experimental groups, we will also perform this experiment with three genes previously identified as drivers of gastric cancer by the TCGA and which also co-occur with CDH1 mutations [Wang et. al., 2014; cBioPortal]. These organoids will then be orthotopically implanted and allowed to grow for up to 12 months. At multiple time points within the 12 month timeframe, mice will be sacrificed and submitted to the NKI Animal Pathology Department for pathological evaluation of gastric tumor growth. This will provide us information on the critical limit regarding the number of mutations needed in order for normal gastric tissue to transition to gastric cancer in a CDH1 mutated background. It will also provide insight on whether the specific mutations, rather than the sheer number of mutations, is important. We additionally hope to correlate our data with malignancy.

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

The aim of our this appendix is to validate candidate drivers of CDH1-mutated gastric cancer using an orthotopic mouse model. The technology for this method is in place in collaboration with the group in the United States that developed the method.

Nature of procedure: This procedure involves fasting the mice overnight (with free access to water) before the procedure to ensure an empty stomach in preparation for implantation of the human organoids the following day. Anaesthesia will be given by isoflurane, according to the NKI "Anaesthesia and pain Relief" SOP, then an 18g, flexible, polyethylene gavage tube of 8cm in length is placed down the throat of a lightly anesthetized mouse until it contacts the stomach mucosa. The smooth passage of the gavage tube will be ensured to prevent injury to the esophagus and stomach during the placement.

Next, a rounded-tip 22g nickel-chromium electrode of a length just greater than the length of the gavage tube is passed down the gavage tube. The tip of the electrocoagulation electrode is rounded to prevent injury to the stomach mucosa. It is also designed so that the rounded tip of the electrode protrudes just beyond the end of the gavage tube. In this manner, the gavage tube protects the gastroesophageal mucosa during passage of the electrode into the stomach. It also provides insulation from the electricity which will be passed down the electrode, except for the rounded, protruding tip. 10W of electricity is then applied to the electrode for 1 second using a Bovie device. This causes a superficial injury to the gastric mucosa. Then, the electrode is removed and organoids in a basement membrane matrix are injected down the gavage tube to the site of superficial injury. In order to avoid movement of the gavage tube as much as possible, we will design a custom restraint platform to hold the mouse in a fixed position during the procedures. In this way, we hope to increase the tumor take rate over the published tumor take rate of ~70%, thereby requiring that we use less animals.

In the original paper, 10^6 cells / 0.2mL of basement membrane matrix were used. As organoids are different than cell lines, we may have to adjust the concentration of organoid cells / 0.2 mL basement membrane matrix in a small pilot experiment (included in the numbers for "Organoid numbers pilot" below). We expect that concentrations in the range of 10^4 to 10^7 organoid cells / 0.2mL of basement membrane matrix will be used in our final experiments. The gavage tube is then removed and the mice are allowed access to water and food ad libitum 2 hours after the procedure. Gastric tumors are expected

form within 12 months and can then be used for further study (pathological examination, DNA, RNA, protein isolation, etc.). Within 12 months, the mice will be sacrificed and submitted to the NKI Mouse Pathology Department for pathological examination to determine whether tumors have formed in the stomach and whether there is any evidence of metastasis.

We will first troubleshoot using human gastric cancer organoids from our in-lab biobank, and a mouse cancer cell line, both of which are known to grow well in vitro (included in the numbers for "Model set-up" below), then we will use this system to test the tumorigenic potential of each of the mutant human and mouse organoid lines in vivo. If we are unable to optimize the system at this point, then we will not proceed.

Frequency and duration of treatment: Each mouse will be subjected to this procedure one time and we expect the procedure to last approximately 15 minutes.

Justification for the selected approach: We have chosen this approach to validate our hits for 3 reasons: 1.) Orthotopic models provide several important advantages over other systems, including physiologically relevant: progression of disease, tumor-host interactions, development of metastases, and organ-specific gene expression [Killion et. al., 1998]. 2.) We aim to identify drivers of gastric cancer, thus we expect the resulting lesions to be very small and minimally invasive. Gastric cancer almost never metastasizes to the subcutaneous space suggesting that this environment is not supportive enough for minimally invasive gastric tumor growth. Therefore, it is highly likely that our engineered organoids would not grow out in the subcutaneous setting and we would miss out on validating important hits. 3.) Although several orthotopic models of gastric cancer are available, we have elected to use the published electrocoagulation-based orthotopic mouse model of gastric cancer, as this model most closely mimics the development of gastric cancer in the human setting and also does not require surgery, resulting in less discomfort to the mice than other available methods, which all require surgery.

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

For the pilot experiments to set up the method, and also for training procedures, we would like to request 10 animals per group. For the pilot experiments, this number is based on institute-wide experience with setting up new mouse cancer models. For the training experiments, we feel that 10 mice per trainee will allow sufficient practice and will also allow us to calculate the success of the method in the hands of each trainee.

For the validation of our candidate genes using engineered organoids, we would like to use group sizes of 9 animals for the control groups and 16 animals for each targeted organoid line.

As a supporting calculation we used a One Sided T-test. We assumed a mean of 1 for the control group (the number of mice with tumors) and 10 for the experimental groups, and a variance of 1 and 5, respectively. However, since the published tumor take rate of this orthotopic model is 70%, and we will require a take rate of >60%, we will therefore use 9 total animals per control group (5 / 60% = 8.33) and 16 total animals per experimental group (10 / 60% = 16.66).

B. The animals

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

Species: mus musculus.

Origin: mice of a genetic background matching the cancer cell line used for the pilot test (own breeding or commercial supplier); NOD-SCID (own breeding or commercial supplier); mice containing conditional alleles for CDH1 and Cas9 (own breeding).

Life stage: adult (both male and female, in proportional quantities).

Justification: Mice are a commonly used animal model in oncology studies due to their short generation time and ease of genetic manipulation. Mice also share a similar organ structure to humans and have

considerable genetic conservation as compared to humans. Furthermore, much research using mice has been conducted, resulting in the availability of many genome-wide data sets and advanced tools for genetic and biological manipulation, including tumor models, cell lines, and xenograft platforms.

Orthotopic model set-up pilot and training:
New orthotopic models of (human or mouse) cancer that have not been used previously in our facility need to be both established and tested to determine the tumor take rate. (We will perform a second pilot experiment (see below) to establish the optimal number of organoid cells needed). For this purpose, we would like to train a total of 5 people, including staff from the Intervention Unit, using 10 mice per trainee (the exact strain will depend on the mouse cancer line which is selected), which we think will allow us sufficient practice in establishing the method, and 1x10^7 cells in 0.2mL of basement membrane matrix, which is 10 times higher than the published number using cell lines [Bhullar et. al., 2010]. For the training, we will use mouse cancer cells, as these cells are expected to develop into gastric cancer within one to two months. Within two months after the procedure, the mice will be sacrificed, and stomachs will be examined for evidence of gastric cancer. **We feel this training pilot is important, since with well-trained technicians, the results of an experiment become more reliable and require less mice.** As the published take rate is 70%, as a measure of competency after training, we will require a take rate of >60%. Since this is a training step and not an experiment which requires extensive controls, we will use only a single organoid line, which we already know grows well in vitro:
1. mouse cancer cells with electrocoagulation

Please note that we do not plain to train all 5 people at once, but training will be done as needed over a period of 5 years. Therefore, we will use a *maximum* of 50 mice for this pilot study and training.

Orthotopic model organoids numbers pilot:
As the previous publication used SNU-16 human gastric cancer cells for their experiments, which is a different cell line and genetic background (and a different species, in the case of our mouse organoid experiments), we may have to adjust the published concentration [Bhullar et. al., 2010] of organoid cells in 0.2 mL basement membrane matrix in a small pilot experiment. We expect that concentrations in the range of 10^4 to 10^7 organoid cells in 0.2mL of basement membrane matrix will be used in our final experiments. In this pilot study, we would like to test the optimal number of cells to use for our mouse and human experiments. For the mouse cell number pilot, we will use a mouse strain of the same background as the cancer cell line that we select for use. This choice will depend on cell line availability, as well as the growth rate of the cell line. For the human cell number pilot, we will use NOD-SCID animals. We will choose a human gastric cancer organoid line from our in-lab biobank that is known to grow well in vitro. We would like to use 16 adult animals per group (10 mice desired / 60% take rate = 16 mice) in 8 groups, covering a range of organoid concentrations:
1. 1x10^7 CDH1 mutant, but non-cancerous, mouse or human gastric organoids
2. mouse cancer cells or human gastric cancer organoids (from our in-lab biobank) at concentration 1
3. mouse cancer cells or human gastric cancer organoids (from our in-lab biobank) at concentration 2
4. mouse cancer cells or human gastric cancer organoids (from our in-lab biobank) at concentration 3
5. mouse cancer cells or human gastric cancer organoids (from our in-lab biobank) at concentration 4
6. 1x10^7 mouse cancer cells or human gastric cancer organoids (from our in-lab biobank) without electrocoagulation
7. 1x10^7 CDH1 mutant, but non-cancerous, mouse or human gastric organoids without electrocoagulation
8. electrocoagulation without organoids
Therefore, we will use 256 mice for this pilot study.

Orthotopic model experimental set-up:
Once we have identified the correct number of organoid cells to use (in 0.2mL basement membrane matrix), we will perform our experiments. Our human (in adult NOD-SCID mice) and mouse (in adult mice containing a conditional CDH1 and Cas9 allele) studies will contain 20 control groups, with 9 mice per group:

1. CDH1 mutant, but non-cancerous, mouse or human gastric organoids at time point 1
2. mouse cancer cells or human gastric cancer organoids (from our in-lab biobank) at time point 1
3. mouse cancer cells or human gastric cancer organoids (from our in-lab biobank) without electrocoagulation at time point 1
4. CDH1 mutant, but non-cancerous, mouse or human gastric organoids without electrocoagulation at time point 1
5. electrocoagulation without organoids at time point 1
6. CDH1 mutant, but non-cancerous, mouse or human gastric organoids at time point 2
7. mouse cancer cells or human gastric cancer organoids (from our in-lab biobank) at time point 2
8. mouse cancer cells or human gastric cancer organoids (from our in-lab biobank) without electrocoagulation at time point 2
9. CDH1 mutant, but non-cancerous, mouse or human gastric organoids without electrocoagulation at time point 2
10. electrocoagulation without organoids at time point 2
11. CDH1 mutant, but non-cancerous, mouse or human gastric organoids at time point 3
12. mouse cancer cells or human gastric cancer organoids (from our in-lab biobank) at time point 3
13. mouse cancer cells or human gastric cancer organoids (from our in-lab biobank) without electrocoagulation at time point 3
14. CDH1 mutant, but non-cancerous, mouse or human gastric organoids without electrocoagulation at time point 3
15. electrocoagulation without organoids at time point 3
16. CDH1 mutant, but non-cancerous, mouse or human gastric organoids at time point 4
17. mouse cancer cells or human gastric cancer organoids (from our in-lab biobank) at time point 4
18. mouse cancer cells or human gastric cancer organoids (from our in-lab biobank) without electrocoagulation at time point 4
19. CDH1 mutant, but non-cancerous, mouse or human gastric organoids without electrocoagulation at time point 4
20. electrocoagulation without organoids at time point 4

And 14-21 experimental groups, at 4 time points per group, with 16 mice per group, which will be made up of various combinations of 1, 2, or 3 mutations (taken from the list of hits from our screen and from the list of drivers previously identified by the TCGA [Wang et. al., 2014; cBioPortal]):

1. (Human or mouse) engineered gastric organoids of genotype 1
2. (Human or mouse) engineered gastric organoids of genotype 2
3. (Human or mouse) engineered gastric organoids of genotype 3
4. (Human or mouse) engineered gastric organoids of genotype 4
5. (Human or mouse) engineered gastric organoids of genotype 5
6. (Human or mouse) engineered gastric organoids of genotype 6
7. (Human or mouse) engineered gastric organoids of genotype 7
8. (Human or mouse) engineered gastric organoids of genotype 8
9. (Human or mouse) engineered gastric organoids of genotype 9
10. (Human or mouse) engineered gastric organoids of genotype 10
11. (Human or mouse) engineered gastric organoids of genotype 11
12. (Human or mouse) engineered gastric organoids of genotype 12
13. (Human or mouse) engineered gastric organoids of genotype 13
14. (Human or mouse) engineered gastric organoids of genotype 14
15. (Human or mouse) engineered gastric organoids of genotype 15
16. (Human or mouse) engineered gastric organoids of genotype 16
17. (Human or mouse) engineered gastric organoids of genotype 17
18. (Human or mouse) engineered gastric organoids of genotype 18
19. (Human or mouse) engineered gastric organoids of genotype 19
20. (Human or mouse) engineered gastric organoids of genotype 20
21. (Human or mouse) engineered gastric organoids of genotype 21

This results in a maximum of 1344 animals per experiment. Since we will perform studies with

both human and mouse targeted organoids, we will use 2688 mice in these studies over 5 years.
The total number of mice requested for the orthotopic section of this proposal is: 2994.

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.

Replacement:
Cell culture experiments will be performed prior to beginning any in vivo work. The results of these experiments will be critically evaluated and in vivo tests will be undertaken only if the cell culture experiments are considered to be sufficiently promising. However, as cancer is a complex disease, and we hope to translate our findings to the clinical setting, our experiments cannot be limited to results from cell culture alone. Organoids, cells, and computer models are not yet sophisticated enough to mimic factors such as the stroma, oxygen supply, the immune system, and metabolism, and therefore cannot yet replace in vivo tumor models.
Reduction:
The proposed number of animals per group (n=9-16 animals) is based on institute-wide experience with these types of experiments, as well as statistical calculations. Further reduction of the number of animals per cohort would decrease the statistical power of the experiments.
Refinement:
By performing pilots to set up the method, to optimize the method, and to properly train personnel, we aim to minimize the discomfort to the animals involved. Additionally, we have elected to use an electrocoagulation-based orthotopic model of gastric cancer which (in contrast to other existing

orthotopic models of gastric cancer) is a non-surgical method and, therefore, causes less discomfort to the mice. The procedure will be performed only once, under anaesthesia, and mice will be closely monitored in the period immediately following completion of the procedure.

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

We realize that the procedures described in this appendix will inevitably cause stress and suffering to the animals. In order to minimize these effects, we will adhere to the general and internationally accepted rules (Code of Practice) of handling lab animals in oncology [Workman et. al., 2010]. Under these rules, the animals will be humanely killed when any humane endpoint is reached. Additionally, for procedures Furthermore, mice are housed in state-of-the-art, individually ventilated cages. As standard procedure, mice in our facility are group-housed and provided with cage enrichment, bedding, and free access to food and water.

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.

Not applicable.

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.

Within the NKI, we have developed a standard operation protocol that describes the most appropriate methods for anaesthesia and analgesia for each procedure. This protocol has been developed by the animal welfare officers of the NKI and may be subject to change when new concepts or ideas about optimal anaesthesia/analgesia evolve. Based on the current protocol, the most appropriate anaesthetic for our application is isoflurane and analgesics are Rimadyl and Temgesic.

I. Other aspects compromising the welfare of the animals

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

Similar to cancer patients, animals carrying tumors in internal organs may develop dysfunction of the involved organs, or other complications due to metastasis (although this has not been seen previously in the orthotopic model that we will use). Although, based on previously published results, we expect that all mice will be sacrificed before a humane endpoint is reached, the mice may undergo moderate discomfort due to tumor growth. Specifically in the case of gastrointestinal tumors, obstruction of the gastro-intestinal tract may occur. Alternatively, the mice may experience weight loss, blood in feces, diarrhea, or obstruction of the GI tract. Should any signs of discomfort be detected, the mouse will be observed daily and the decision to sacrifice the mouse will be made with the help of the NKI animal welfare officers. If a humane endpoint is reached, the mouse will be sacrificed immediately

Explain why these effects may emerge.

These effects are an unavoidable consequence of tumor growth.

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

In general, the effects on the wellbeing of the mice due to tumor growth cannot be completely avoided. In order to minimize the burden on the mice, they will be monitored weekly for signs of distress (hunched back, lack of grooming, blood in feces, signs of infection, significant weight loss) and killed when any of the humane endpoints described below are met. Nevertheless, unforeseen complications may arise. In such cases, we will attempt to find solutions which minimize the impact of unforeseen complications, for example, by providing easy access to food (mush-feeding), taking into account the humane endpoints below.

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 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 general, the most important humane endpoints that apply are:

- Weight loss of more than 20% of the initial body weight, measured from the start of treatment of adult animals.
- Severe abnormal breathing.
- Evidence of abnormal stool (for example, blood in the stool).
- Severe abnormal behavior (for example: lack of grooming, humched posture, pilo-erection).

Indicate the likely incidence.

Since the published tumor take rate of our orthotopic mouse model of human or mouse cancer is 70%, we expect <70% of our non-control mice to develop gastric cancer. However, based on previously published results [Shimada et. al., 2012; Humar et. al., 2009], we do not expect humane endpoints to be reached.

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').

Our orthotopic mouse model of human or mouse gastric cancer involves anaesthetizing the mouse, oral gavage, and application of a light electrical current, prior to administration of our cells of interest in a basement membrane matrix. Not all of the mice will be treated with potentially tumorigenic organoids, but since all mice in this appendix will be subjected to the electrocoagulation procedure, which requires anaesthesia, we expect that all mice in this appendix will experience moderate discomfort.

Additional discomfort due to procedures:
Simple but frequent handlings, like weighing: mild discomfort.

Therefore, we expect that mice under this appendix will experience mild, moderate, and severe discomfort in: 0, 100, and 0% of the cases, respectively.

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.

In order to do a full pathology exam on the tumors, and necropsy on the mice (to look for metastases), it is necessary to remove the affected organ(s). Therefore, the mice will need to be sacrificed at the end of the experiment.

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	Subcutaneous models of gastric cancer

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.

We would like to test organoid lines that are engineered to contain mutations in candidate drivers of gastric cancer, and which are suspected to be more malignant, in a subcutaneous model. This is an important step in our project, as it will provide us with information regarding whether any of the driver mutations that we identify lead to particularly aggressive gastric cancer. This is necessary information if we are to advise patients on whether or not to undergo gastrectomy or to follow a more conservative treatment regimen. This step will allow us not only to confirm the tumorigenicity of these engineered organoid lines, but it will also allow us to look at the invasiveness of the lines. Additionally, it will provide us with a tool to grow a larger number of tumor cells for further genetic and biochemical analyses. And, since we expect the engineered organoid lines that we select for this experiment to be more malignant, the subcutaneous model will cause the least amount of discomfort to the mice since the tumors that form will be visible under the skin and can be closely monitored.

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

Nature of procedure: This procedure involves injecting organoids of interest subcutaneously into an anaesthetized mouse. Anaesthesia will be given by isoflurane, according to the NKI "Anaesthesia and Pain Relief SOP" and organoids will be injected into the rear flank. Mice will be sacrificed within 12 months of injection, or once the tumor reaches a size of 1500mm^3, whichever occurs first. The resulting tumors will be submitted to the NKI Mouse Pathology Department for pathological examination.

Frequency and duration of treatment: Each mouse will be subjected to this procedure one time and we expect the procedure to last not more than 15 minutes.

Justification for the selected approach: We have chosen this approach for three reasons 1.) it is a well-established and well-accepted model of tumor growth, 2.) it will allow us to test the ability of our targeted organoids to form tumors outside of their tissue of origin, and 3.) it will allow us to do so with minimal discomfort to the mice since the tumors that may form are visible externally, allowing us to closely monitor tumor growth.

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

For the pilot experiment to set up the method, we would like to request 10 animals per group. This number is based on institute-wide experience with setting up new mouse cancer models.

For the experiments to test the malignancy of our engineered organoid lines, we would like to use group sizes of 5 animals for the control groups and 10 animals for each targeted organoid line.

As a supporting calculation we used a One Sided T-test. We assumed a mean of 1 for the control group (the number of mice with tumors) and 10 for the experimental groups, and a variance of 1 and 5, respectively.

B. The animals

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

Species: mus musculus.

Origin: NOD/SCID (own breeding or commercial supplier); mice containing conditional alleles for CDH1 and Cas9 (own breeding).

Life stage: adult (both male and female, in proportional quantities).

Justification: Mice are a commonly used animal model in oncology studies due to their short generation time and ease of genetic manipulation. Mice also share a similar organ structure to humans and have considerable genetic conservation as compared to humans. Furthermore, much research using mice has been conducted, resulting in the availability of many genome-wide data sets and advanced tools for genetic and biological manipulation, including tumor models, cell lines, and xenograft platforms.

Subcutaneous model set-up pilot and organoid numbers pilot:
New models of (human or mouse) cancer that have not been used previously in our facility need to be tested to establish the tumor take rate and the optimal number of cells to inject. While the subcutaneous method is commonly used in our institute, it has not yet been tested for use with gastric organoids. Based on the numbers reported in a previously published model [Drost et. al., 2015], we will inject 2x10^5 to 2x10^7 gastric cancer organoids, which are known to grow well in vitro, in 100ul total volume (1:1 basement membrane matrix:media). We will use a range, rather than simply using the previously published number, as the previously published model was performed with colorectal organoids. Therefore, we would like to ensure that the optimal number of organoids is identified for use with gastric cancer. For this purpose, we would like to use 10 adult NOD-SCID mice per group:
1. 2x10^7 CDH1 mutant, but non-cancerous, human gastric organoids
2. human gastric cancer organoids (from our in-lab biobank) at concentration 1
3. human gastric cancer organoids (from our in-lab biobank) at concentration 2
4. human gastric cancer organoids (from our in-lab biobank) at concentration 3
5. 2x10^7 human gastric cancer organoids (from our in-lab biobank) without electrocoagulation
6. vehicle injection without organoids

Therefore, we will use 60 mice for this pilot study.

Subcutaneous model experimental set-up:
Once we have identified the correct number of organoid cells to use (in 0.1mL basement membrane

matrix), we will perform our experiments. A typical human (in adult NOD-SCID mice) or mouse (in adult mice containing a conditional CDH1 and Cas9 allele) study will contain 3 control groups, with 5 mice per group:
1. CDH1 mutant, but non-cancerous, (human or mouse) gastric organoids
2. human gastric cancer organoids (from our in-lab biobank) or mouse cancer cells
3. vehicle injection without organoids

and 14-21 experimental groups, with 10 mice per group, which will be made up of various combinations of one, two, three, or four mutations (taken from the results of the orthotopic model validation experiment in Appendix 2, or published candidate drivers of HDGC), in a CDH1 mutated background:
1. (Human or mouse) engineered gastric organoids of genotype 1
2. (Human or mouse) engineered gastric organoids of genotype 2
3. (Human or mouse) engineered gastric organoids of genotype 3
4. (Human or mouse) engineered gastric organoids of genotype 4
5. (Human or mouse) engineered gastric organoids of genotype 5
6. (Human or mouse) engineered gastric organoids of genotype 6
7. (Human or mouse) engineered gastric organoids of genotype 7
8. (Human or mouse) engineered gastric organoids of genotype 8
9. (Human or mouse) engineered gastric organoids of genotype 9
10. (Human or mouse) engineered gastric organoids of genotype 10
11. (Human or mouse) engineered gastric organoids of genotype 11
12. (Human or mouse) engineered gastric organoids of genotype 12
13. (Human or mouse) engineered gastric organoids of genotype 13
14. (Human or mouse) engineered gastric organoids of genotype 14
15. (Human or mouse) engineered gastric organoids of genotype 15
16. (Human or mouse) engineered gastric organoids of genotype 16
17. (Human or mouse) engineered gastric organoids of genotype 17
18. (Human or mouse) engineered gastric organoids of genotype 18
19. (Human or mouse) engineered gastric organoids of genotype 19
20. (Human or mouse) engineered gastric organoids of genotype 20
21. (Human or mouse) engineered gastric organoids of genotype 21

This results in a maximum of 225 animals per experiment. Since we will perform studies with both human and mouse targeted organoids, we will use 450 mice in these studies over 5 years.
The total number of mice requested for the subcutaneous section of this proposal is: 510.

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.

Replacement:
Cell culture experiments will be performed prior to beginning any in vivo work. The results of these experiments will be critically evaluated and in vivo tests will be undertaken only if the cell culture experiments are considered to be sufficiently promising. However, as cancer is a complex disease, and

we hope to translate our findings to the clinical setting, our experiments cannot be limited to results from cell culture alone. Organoids, cells, and computer models are not yet sophisticated enough to mimic factors such as the stroma, oxygen supply, the immune system, and metabolism, and therefore cannot yet replace in vivo tumor models.
Reduction:
The proposed number of animals per group (n=5-10 animals) is based on institute-wide experience with these types of experiments, as well as statistical calculations. Further reduction of the number of animals per cohort would decrease the statistical power of the experiments.
Refinement:
We have elected to perform this analysis in a subcutaneous, rather than an orthotopic model, since we expect the engineered organoid lines that we select for this experiment to be more malignant. The subcutaneous model will cause the least amount of discomfort to the mice since the tumors that form will be visible under the skin. Therefore, we can monitor tumor growth carefully, which would not be possible in an orthotopic model where the tumors form in an internal organ and may, for example, result in obstruction of the GI tract. Additionally, injections will be performed under once only once, under anaesthesia, and in an appropriate volume. Animals will be closely monitored during the immediate post injection period and tumor growth will be measured every week.

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

We realize that the procedures described in this appendix will inevitably cause stress and suffering to the animals. In order to minimize these effects, we will adhere to the general and internationally accepted rules (Code of Practice) of handling lab animals in oncology [Workman et. al., 2010]. Under these rules, the animals will be humanely killed when any humane endpoint is reached. Furthermore, mice are housed in state-of-the-art, individually ventilated cages. As standard procedure, mice in our facility are group-housed and provided with cage enrichment, bedding, and free access to food and water.

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.

Not applicable.

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.

Within the NKI, we have developed a standard operation protocol that describes the most appropriate methods for anaesthesia and analgesia for each (surgical) procedure. This protocol has been developed by the animal welfare officer of the NKI and may be subject to change when new concepts or ideas about optimal anaesthesia/analgesia evolve. Based on the current protocol, the most appropriate anaesthetic for our application is isoflurane and analgesic is Rimadyl.

I. Other aspects compromising the welfare of the animals

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

Similar to cancer patients, animals carrying tumors implanted subcutaneously may develop complications due to growth of the primary tumor or due to metastasis. Although all mice will be sacrificed as soon as a humane endpoint is reached, the mice may undergo mild to moderate discomfort due to tumor growth. Should any signs of discomfort be detected, the mouse will be observed daily and the decision to sacrifice the mouse will be made with the help of the NKI animal welfare officers. If a humane endpoint is reached, the mouse will be sacrificed immediately

Explain why these effects may emerge.

These effects are an unavoidable consequence of tumor growth.

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

In general, the effects on the wellbeing of the mice due to tumor growth cannot be completely avoided. In order to minimize the burden on the mice, they will be monitored for signs of distress (hunched back, lack of grooming, blood in feces, signs of infection, significant weight loss) and killed when any of the humane endpoints described below are met. Nevertheless, unforeseen complications may arise. In such cases, we will attempt to find solutions which minimize the impact of unforeseen complications, for example, by providing easy access to food (mush-feeding), taking into account the humane endpoints below.

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 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 general, the most important humane endpoints that apply are:

- Weight loss of more than 20% of the initial body weight, measured from the start of treatment of adult animals.
- Tumor mass greater than 10% of the body weight.
- Severe abnormal breathing.
- Severe abnormal behavior (for example: lack of grooming, humched posture, pilo-erection).
- Subcutaneous tumor of greater than 1500mm^3

Indicate the likely incidence.

For the subcutaneous model, we expect that only a subset of the mutations tested will lead to tumor formation. If we estimate that 50% of the mice will form tumors of various sizes, then it is possible that 50% of our mice may reach a tumor mass of >10% of the body weight or a tumor volume of 1500mm^3.

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').

Our subcutaneous mouse model of human or mouse gastric cancer involves the subcutaneous injection of cells and the development of visible tumors under the skin. Therefore, those mice which develop tumors may experience mild to moderate discomfort due to subcutaneous tumor growth. Although it is not possible to know which organoid lines will form subcutaneous tumors without testing this directly, we estimate that 50% of the organoid lines that we test will be able to form subcutaneous tumors, which results in up to 50% of the mice in this appendix experiencing mild to moderate discomfort. The remaining 50% of the mice will experience mild discomfort due to the subcutaneous implantation procedure, which requires anaesthesia. To prevent unnecessary suffering, all mice will be sacrificed in the event that the tumor reaches 1500mm^3.

Additional discomfort due to procedures:
Simple but frequent handlings, like weighing and tumor volume measurements: mild discomfort.

Therefore, we expect that mice under this appendix will experience mild, moderate, and severe discomfort in: 50, 50, and 0% of the cases, respectively.

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.

In order to do a full pathology exam on the tumors, and necropsy on the mice (to look for metastases), it is necessary to remove the affected organ(s). Therefore, the mice will need to be sacrificed at the end of the experiment.

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

7.

Dierexperimentencommissie NKI

[redacted]

NETHERLANDS CANCER INSTITUTE
ANTONI VAN LEEUWENHOEK

DEC advies aan CCD

A. Algemene gegevens over de procedure

1. Aanvraagnummer:
1. Titel van het project: Screening for drivers of gastric cancer
2. Titel van de NTS: Screening voor drijvers van maagkanker

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

4. Contactgegevens DEC:
   - naam DEC:
   - telefoonnummer contactpersoon:
   - e-mailadres contactpersoon:

5. Adviestraject (data dd-mm-jjjj):
   ☒ ontvangen door DEC: 04-10-2016
   ☐ aanvraag compleet
   ☒ in vergadering besproken: 12-10-2016 en 10-01-2017
   ☐ anderszins behandeld
   ☒ termijnonderbreking(en) van 19-10-2016 tot 02-01-2017 en 30-01-2017 tot 02-02-2017
   ☐ besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen
   ☒ aanpassing aanvraag: 02-01-2017 en 02-02-2017
   ☒ advies aan CCD: 20-02-2017

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

7. Eventueel horen van aanvrager: n.v.t.
   - Datum:
   - Plaats:
   - Aantal aanwezige DEC-leden:
   - Aanwezige (namens) aanvrager:
   - Gestelde vraag / vragen:
   - Verstrekt(e) antwoord(en)

   - Vragen (datum: 19-10-2016) en *antwoorden* (datum: 02-01-2017):
   - 1.3.: de DEC acht de titel niet representatief voor de aanvraag. De organoids staan niet centraal in deze aanvraag. Die vormen niet meer dan een tool bij het zoeken naar genen die (als een soort second hit) betrokken zouden kunnen zijn bij HDGC. De DEC ziet de screen die zich richt op maagkanker als de kern van deze aanvraag. Dat zou ook in de titel tot uiting moeten komen.
   - *Aangepast.*

- 2.1.: de DEC denkt dat dit geen translationeel onderzoek is. “Translational or applied research” zou dan uitgevinkt moeten worden.
- *Aangepast.*
- 3.1.: de DEC heeft de onderzoeker gevraagd beter te onderbouwen waarom hij denkt dat één “extra hit” naast CDH1 voldoende is voor het ontstaan van maagkanker (HDGC).
- *De onderzoeker heeft dit nader toegelicht in het antwoord op vraag 3.1 en 3.4.1.*
- 3.1.: De DEC heeft haar zorgen geuit over de haalbaarheid van dit onderzoek. Welke aanwijzingen zijn er dat de screen in deze vorm werkt?
- *De onderzoeker heeft dit nader toegelicht in het antwoord op vraag 3.1.*
- 3.2.: De DEC zou graag zien dat de directe doelen meer worden benadrukt. Er ligt nu veel nadruk op “the overarching goal”.
- *De onderzoeker heeft de directe doelen opgenomen in de tekst van het antwoord op deze vraag.*
- 3.4.1.: De DEC is nog niet geheel overtuigd door uw argumenten voor de orthotope benadering om de resultaten van de screen te valideren.
- *De onderzoeker heeft aanvullende argumenten opgenomen in de tekst van het antwoord op vraag 3.4.1.*
- 3.4.: de DEC verzoekt de onderzoeker het doel van het gebruik van de methode met de magneet te verduidelijken.
- *De onderzoeker heeft een toelichting toegevoegd.*
- 3.4.: de DEC verzoekt de onderzoeker een duidelijk stappenplan dan wel schema op te nemen, zodat de volgorde van de experimenten inzichtelijker wordt.
- *De onderzoeker heeft een flowchart toegevoegd.*
- **Appendix 1:**
- De DEC heeft de onderzoeker tal van redactionele en praktische suggesties aan de hand gedaan.
- *De onderzoeker heeft veel van die suggesties gevolgd.*
- 2B (dieren): de DEC verzoekt de onderzoeker aan te geven of mannetjes en vrouwtjes in evenredige aantallen zullen worden gebruikt. Indien dat niet zo is, wat zijn dan de redenen daarvoor? Kunt u ook aangeven om welke stam(men) het gaat en of het klopt dat dit commerciële dieren zullen zijn?
- *de onderzoeker heeft in de aanvraag tekst opgenomen over het geslacht van de te gebruiken dieren en over de te gebruiken stammen en hun herkomst.*
- 2B: (in vivo screen training): de DEC acht de aantallen dieren aangevraagd voor de training uit balans met het totale aantal en vraagt de onderzoeker of het zou volstaan om 2 à 3 personen te trainen.
- *Het aantal te trainen personen is teruggebracht naar maximaal 4.*
- 2B: (in vivo screen experimental set-up): de DEC verzoekt de onderzoeker te heroverwegen of de eerste 6 groepen met buffer noodzakelijk zijn en of eventueel kan worden volstaan met het laatste tijdstip.

- *De onderzoeker heeft uitgelegd dat dit in principe kan, maar dat het er toe zou leiden dat interessante tussentijdse resultaten pas na lange tijd (zelfs tot een jaar) vergeleken kunnen worden met de negatieve controlegroep, omdat die ene negatieve conrolegroep tot het eind in de proef moet blijven. Dit is ongewenst.*

- D: de DEC verzoekt de onderzoeker het in vitro voorwerk duidelijker te beschrijven.
- *Aangepast.*

- **Appendix 2**
- De DEC heeft de onderzoeker tal van redactionele en praktische suggesties aan de hand gedaan.
- *De onderzoeker heeft veel van die suggesties gevolgd.*

- Algemeen: wijzigingen in de voorgaande bijlage die ook van toepassing zijn in bijlage 2, dienen ook hier te worden doorgevoerd.

- 2B (dieren): de DEC verzoekt de onderzoeker aan te geven of mannetjes en vrouwtjes in evenredige aantallen zullen worden gebruikt. Indien dat niet zo is, wat zijn dan de redenen daarvoor? Kunt u ook aangeven om welke stam(men) het gaat en of het klopt dat dit commerciële dieren zullen zijn?
- *de onderzoeker heeft in de aanvraag tekst opgenomen over het geslacht van de te gebruiken dieren en over de te gebruiken stammen en hun herkomst.*
- B: (orthotopic model training): de DEC vraagt de onderzoeker of het volstaat om minder personen te trainen.
- *Het aantal te trainen personen is teruggebracht naar maximaal 5.*

- Appendix 3:
- De DEC heeft de onderzoeker tal van redactionele en praktische suggesties aan de hand gedaan.
- *De onderzoeker heeft veel van die suggesties gevolgd.*

- Algemeen: wijzigingen in voorgaande bijlagen die ook van toepassing zijn in bijlage 3, dienen ook hier te worden doorgevoerd.

- B (dieren): de DEC verzoekt de onderzoeker aan te geven of mannetjes en vrouwtjes in evenredige aantallen zullen worden gebruikt. Indien dat niet zo is, wat zijn dan de redenen daarvoor? Kunt u ook aangeven om welke stam(men) het gaat en of het klopt dat dit commerciële dieren zullen zijn?
- *de onderzoeker heeft in de aanvraag tekst opgenomen over het geslacht van de te gebruiken dieren en over de te gebruiken stammen en hun herkomst.*

- NTS:
- In het algemeen lijkt het de DEC een te lange en te ingewikkelde NTS, waarbij het taalgebruik beter zou kunnen worden afgestemd op de doelgroep.
- algemeen: wijzigingen in het project naar aanleiding van vragen van de DEC, dienen ook te worden doorgevoerd in de NTS.
- *De onderzoeker heeft de NTS aangepast.*

- 2^{e} ronde vragen (datum: 30-01-2017) en *antwoorden* (datum:0 2-02-2017)

- de DEC heeft de antwoorden van de eerste ronde en de bijgestelde versies van de projectaanvraag, bijlagen en NTS besproken en in reactie daarop de aanvrager nog een aantal redactionele en praktische suggesties aan de hand gedaan. Dit betrof niet opgevolgde redactionele suggesties uit de eerste ronde en het beantwoorden van vraag 2E in de bijlagen met “niet van toepassing”.
- *Alle suggesties zijn door de aanvrager verwerkt.*

8. 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. Geen van de DEC-leden is betrokken bij deze projectaanvraag.

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 1 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.
   In dit project zal om te beginnen een genetische screen bij muizen uitgevoerd worden om genen te vinden die in combinatie met het gen CDH1 betrokken zijn bij het ontstaan van een erfelijke vorm van maagkanker (Erfelijke Diffuse Maagkanker: HDGC). De hypothese is dat een mutatie in een ander relevant gen (een second hit naast CDH1), de kans op het ontstaan van maagkanker aanzienlijk vergroot en mede bepaalt hoe agressief de tumor zal zijn. De kandidaatgenen die uit de screening naar voren komen zullen in een orthotoop model (dus in de maag) bij muizen worden gevalideerd. Na die validatie worden in een subcutaan muismodel de invasieve eigenschappen van de verschillende tumoren onderzocht. Dit alles moet uiteindelijk bijdragen aan het ontwikkelen van een test waarmee deze erfelijke vorm van maagkanker vroegtijdig kan worden opgespoord bij mensen waarvan bekend is dat ze een CDH1 mutatie hebben. Het ontwerpen en valideren van de test maakt geen deel uit van deze projectaanvraag.
   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 vinden van genen die samen met CDH1 betrokken zijn bij het ontstaan en verloop van een erfelijke vorm van maagkanker. De hypothese is dat een mutatie in een ander relevant gen (een second hit naast CDH1), nodig is om maagkanker te laten ontstaan en mede bepaalt hoe agressief de tumor zal zijn. De daadwerkelijke invloed van de in de screening gevonden genen op het ontstaan van kanker en op de eigenschappen van de tumor zal worden gevalideerd en nader onderzocht. Het uiteindelijke doel is om langs deze weg informatie te krijgen die behulpzaam kan zijn bij het opzetten van een test voor erfelijke maagkanker bij mensen waarvan bekend is dat ze een CDH1 mutatie hebben. Het opzetten en valideren van die test maakt geen deel uit van dit project. Het verband tussen het directe doel en het uiteindelijke doel is dus niet direct aanwezig binnen dit project, het betreft immers fundamenteel onderzoek, maar wel reëel. Het doel van deze projectaanvraag is gerechtvaardigd binnen de context van het onderzoeksveld.

5. De belangrijkste belanghebbenden in deze projectaanvraag zijn de proefdieren, de onderzoekers en de doelgroep/patiënten.
   Voor de proefdieren geldt dat hun welzijn en integriteit worden aangetast. De dieren zullen beperkt worden in hun natuurlijke gedrag en gedurende de proeven zullen de dieren stress ondervinden en pijn ondergaan. Uiteindelijk zullen ze in het kader van het onderzoek gedood worden. De dieren hebben er belang bij hiervan gevrijwaard te blijven.
   Voor de onderzoekers geldt dat ze belangrijke nieuwe wetenschappelijke inzichten kunnen publiceren, hetgeen vaak de sleutel is voor het verkrijgen van nieuwe onderzoeksmiddelen en -mogelijkheden. Naar de mening van de DEC dient dat geen rol te spelen in de ethische afweging over de toelaatbaarheid van het gebruik van proefdieren, maar is het ook niet bezwaarlijk als nieuwsgierigheid en ambitie belangrijke drijfveren zijn voor onderzoekers. Voor de rechtvaardiging van dit onderzoek gaat het uiteindelijk echter om de vraag of het 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 de dragers van een CDH1 mutatie is dit onderzoek van belang, omdat het op termijn kan bijdragen aan een verbetering van de mogelijkheden om de kans op een agressieve vorm van erfelijke maagkanker te voorspellen en de maagkanker in een vroeg stadium op te sporen en te behandelen, waardoor de patiënt uitzicht heeft op genezing of een langere overlevingstijd met een beter kwaliteit van leven. Het probleem is op dit moment namelijk dat deze vorm van maagkanker vaak pas in een laat stadium ontdekt wordt, waardoor de tumor al niet meer te behandelen is.

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. Intrinsiek is dit project naar de mening van de DEC wel vrij risicovol, in die zin dat al vroeg in het project zou kunnen blijken dat de gekozen benadering niet werkt, maar dat is te rechtvaardigen gezien het belang van het potentieel hiermee te verkrijgen inzicht. Bovendien is op de juiste punten in het project een go/no go moment ingebouwd, zodat het project tijdig kan worden gestopt. De onderzoeker heeft na vragen daarover van de DEC zijn aanpak nader toegelicht. De DEC acht de wetenschappelijk

keuzes die de aanvrager maakt verdedigbaar. 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. Voor het grootste deel van de dieren (94%) is het ongerief maximaal matig. Dit ongerief wordt hoofdzakelijk bepaald door (chirurgische) ingrepen onder anesthesie die tot doel hebben tumoren te induceren. Tumorgroei en metastasering zullen in dit project niet leiden tot ernstig ongerief, omdat het voor de doelstelling niet nodig is tumoren lang en tot grote omvang te laten groeien. Er worden strikte humane eindpunten gehanteerd, maar de verwachting is dat slechts een klein deel van de dieren (bijlage 3: subcutane tumoren) een humaan eindpunt zal bereiken, omdat de omvang van de tumor anders te groot zou worden. Op dat moment is echter van ernstig ongerief nog geen sprake. Bij de overige dieren (6%) zal het ongerief licht zijn, omdat de subcutane tumor niet aanslaat of slechts zeer beperkt groeit en de handelingen om de subcutane tumor te induceren niet meer dan licht ongerief veroorzaken.
    Het cumulatief ongerief voor de dieren is dus juist ingeschat als matig voor 94% van de dieren, en licht voor 6% 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 aangetast door het induceren van orthotope en subcutane tumoren. Dit leidt in de eerste plaats tot een matige aantasting van het welzijn, maar het valt niet uit te sluiten dat dit ook invloed heeft op het gedrag en zelfredzaamheid van de dieren. Naar het oordeel van de DEC liggen de gehanteerde humane eindpunten ruim voor het moment waarop dergelijke aantastingen van de integriteit problematische vormen aannemen. De commissie is daarom van mening dat er sprake is van een lichte 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 tumorontwikkeling veel ongerief veroorzaakt. Dit is op basis van eerdere ervaringen en gegevens in de literatuur ingeschat. In bijlage 3 zal naar schatting de helft van de dieren waarbij de subcutane tumor aanslaat, een humaan eindpunt bereiken, omdat de tumor de vooraf bepaalde maximale omvang van 1500mm^3 overschrijdt. Op dat moment is van ernstig ongerief echter nog geen sprake. 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. De genetische screening vindt plaats met genen die geselecteerd zijn door onderzoek bij maagkankerpatiënten met een CDH1 mutatie. De orthotope validatie en het subcutane onderzoek zijn noodzakelijk. De complexe interactie tussen de tumor en het immuunsysteem en tussen de tumor en het omliggende weefsel zijn niet *in vitro* na te bootsen. 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 gehanteerde screeningstechniek (met een lentivirale gRNA library) maakt het mogelijk om bijna duizend genen tegelijk te testen met een naar verhouding klein aantal dieren. Voor de volgende stap wordt een selectie gemaakt van een veel kleiner aantal genen waarmee vervolgens verder onderzoek wordt gedaan. De onderzoekers hanteren ook in de rest van het onderzoek 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.

16. Het project is in overeenstemming met de vereiste van de verfijning van dierproeven. De dieren worden niet langer dan noodzakelijk in het experiment gehouden en er worden adequate humane eindpunten gehanteerd. Daarbij wordt de "Code of Practice" voor het kankeronderzoek gevolgd. Voor de inductie van de orthotope tumoren worden verfijnde, niet chirurgische methoden benut. 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 gebruik maken van zowel mannelijke, als vrouwelijke dieren in gelijke hoeveelheden.

19. De dieren zullen in het kader van het project gedood worden. Dit is noodzakelijk om weefsels en organen na afloop te kunnen uitnemen voor verder onderzoek en om te voorkomen dat de zich verder ontwikkelende tumor ongerief zal gaan veroorzaken. 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 vinden van genen die samen met CDH1 betrokken zijn bij het ontstaan en verloop van een erfelijke vorm van maagkanker. De hypothese is dat een mutatie in een ander relevant gen (een second hit naast CDH1), nodig is om maagkanker te laten ontstaan en mede bepaalt hoe agressief de tumor zal zijn. De daadwerkelijke invloed van de in de screening gevonden genen op het ontstaan van kanker en op de eigenschappen van de tumor zal worden gevalideerd en nader onderzocht. Het uiteindelijke doel is om langs deze weg informatie te krijgen die behulpzaam kan zijn bij het opzetten van een test voor erfelijke maagkanker bij mensen waarvan bekend is dat ze een CDH1 mutatie hebben. Bij mensen met een mutatie in dit gen wordt op dit moment vaak pas in een relatief laat stadium vastgesteld dat ze maagkanker hebben, waardoor de tumor al niet meer behandeld kan worden. Een verbetering van de mogelijkheden om de kans op een agressieve vorm van erfelijke maagkanker te voorspellen en de maagkanker in een vroeg stadium op te sporen en te behandelen, waardoor de patiënt uitzicht heeft op genezing of een langere overlevingstijd met een beter kwaliteit van leven, acht de DEC 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]

Centrale Commissie Dierproeven

8.

> Retouradres Postbus 20401 2500 EK Den Haag

Nederlands Kanker Instituut
[redacted]
Postbus 90203
1066 CX 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
AVD301002017876

Bijlagen
2

Datum 21 februari 2017
Betreft Ontvangstbevestiging aanvraag projectvergunning Dierproeven

Geachte [redacted],

Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 20 februari 2017. Het gaat om uw project "Screening for drivers of gastric cancer". Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD301002017876. 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:
21 februari 2017
Aanvraagnummer:
AVD301002017876

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlagen:
- Gegevens aanvraagformulier
- Factuur

Datum:
21 februari 2017
Aanvraagnummer:
AVD301002017876

Gegevens aanvrager

Uw gegevens

Deelnemersnummer NVWA:	30100
Naam instelling of organisatie:	Nederlands Kanker Instituut
Naam portefeuillehouder of diens gemachtigde:	[redacted]
KvK-nummer:	40530817
Straat en huisnummer:	Plesmanlaan 121
Postbus:	90203
Postcode en plaats:	1066 CX AMSTERDAM
IBAN:	NL71DEUT0626343534
Tenaamstelling van het rekeningnummer:	Stichting Het Nederlands Kanker Instituut – Antoni van Leeuwenhoek Ziekenhuis

Gegevens verantwoordelijke onderzoeker

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

Datum:
21 februari 2017
Aanvraagnummer:
AVD301002017876

Gegevens plaatsvervangende verantwoordelijke onderzoeker

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

Gegevens verantwoordelijke uitvoering proces

Naam:	[redacted]
Functie:	[redacted]
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 maart 2017
Geplande einddatum:	1 maart 2022
Titel project:	Screening for drivers of gastric cancer
Titel niet-technische samenvatting:	Screening voor drijvers van menselijke kanker
Naam DEC:	NKI
Postadres DEC:	t.a.v. [redacted]; Postbus 90203; 1006 BE Amsterdam
E-mailadres DEC:	[redacted]

Betaalgegevens

De leges bedragen:	€ 1.541,-
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:	[redacted]
Plaats:	Amsterdam
Datum:	20 februari 2017

Centrale Commissie Dierproeven

10.

> Retouradres Postbus 20401 2500 EK Den Haag

Nederlands Kanker Instituut
[redacted]
Postbus 90203
1066 CX 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
AVD301002017876
Bijlagen
1

13 MRT 2017

Datum 10 maart 2017
Betreft Beslissing aanvraag projectvergunning Dierproeven

Geachte [redacted],

Op 20 februari 2017 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Screening for drivers of gastric cancer" met aanvraagnummer AVD301002017876. Wij hebben uw aanvraag beoordeeld.

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

U kunt met uw project "Screening for drivers of gastric cancer" starten. De vergunning wordt afgegeven van 14 maart 2017 tot en met 1 maart 2022.

Overige wettelijke bepalingen blijven van kracht.

Procedure
Bij uw aanvraag heeft u een advies van de Dierexperimentencommissie NKI gevoegd. Dit advies is opgesteld op 20 februari 2017. Bij de beoordeling van uw aanvraag is dit advies betrokken overeenkomstig artikel 10a, lid 3 van de wet.
Wij kunnen ons vinden in de inhoud van het advies van de Dierexperimentencommissie. Dit advies van de commissie nemen wij over, inclusief de daaraan ten grondslag liggende motivering. Er worden aanvullende algemene voorwaarde(n) gesteld.
Het DEC-advies en de in de bijlage opgenomen beschrijving van de artikelen van de wet- en regelgeving zijn de grondslag van dit besluit.

Datum:
10 maart 2017
Aanvraagnummer:
AVD301002017876

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

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

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

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

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

Centrale Commissie Dierproeven
namens deze:

[redacted]

ir. G. de Peuter
Algemeen Secretaris

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

Centrale Commissie Dierproeven

Projectvergunning

gelet op artikel 10a van de Wet op de Dierproeven

Verleent de Centrale Commissie Dierproeven aan

Naam:	Nederlands Kanker Instituut
Adres:	Postbus 90203
Postcode en plaats:	1066 CX AMSTERDAM
Deelnemersnummer:	30100

deze projectvergunning voor het tijdvak 14 maart 2017 tot en met 1 maart 2022, voor het project "Screening for drivers of gastric cancer" met aanvraagnummer AVD301002017876, volgens advies van Dierexperimentencommissie NKI. Er worden aanvullende algemene voorwaarde(n) gesteld.
De functie van de verantwoordelijk onderzoeker is [redacted]. 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 februari 2017
2 de bij het aanvraagformulier behorende bijlagen:
a Projectvoorstel, zoals ontvangen per digitale indiening op 20 februari 2017;
b Niet-technische Samenvatting van het project, zoals ontvangen per brief op 10 maart 2017;
c Advies van dierexperimentencommissie d.d. 20 februari 2017, ontvangen op 20 februari 2017.

Naam proef	Diersoort/ Stam	Aantal dieren	Ernst	Opmerkingen
3.4.4.1 In vivo screen for drivers of gastric cancer				
	Muizen (Mus musculus) /	580	100% Matig	
3.4.4.2 Orthotopic models of gastric cancer				
	Muizen (Mus musculus) /	2.994	100% Matig	
3.4.4.3 Subcutaneous models of gastric cancer				
	Muizen (Mus musculus) /	510	50% Matig 50% Licht	

Aanvraagnummer:
AVD301002017876

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

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

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

Aanvraagnummer:
AVD301002017876

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.