	Inventaris Wob-verzoek W17-09								
		wordt verstrekt				**weigeringsgronden**			
nr.	**document**	**reeds openbaar**	**niet**	**geheel**	**deels**	**10.1.c**	**10.2.e**	**10.2.g**	**11.1**
	NTS20171264								
1	Aanvraagformulier				x		x	x	
2	Projectvoorstel oud				x		x	x	
3	Niet-technische samenvatting oud			x					
4	Bijlage beschrijving dierproeven 1 oud				x		x	x	
5	Bijlage beschrijving dierproeven 2 oud				x		x	x	
6	Bijlage beschrijving dierproeven 3 oud				x		x	x	
7	DEC-advies				x			x	
8	Ontvangstbevestiging				x		x	x	
9	Verzoek aanvulling aanvraag				x		x	x	
10	Reactie aanvulling aanvraag			x					
11	Projectvoorstel nieuw				x		x	x	
12	Bijlage beschrijving dierproeven 1 nieuw				x		x	x	
13	Bijlage beschrijving dierproeven 2 nieuw				x		x	x	
14	Bijlage beschrijving dierproeven 3 nieuw				x		x	x	
15	Niet-technische samenvatting nieuw	x							
16	Advies CCD		x						x
17	Beschikking en vergunning				x		x	x	

1.

Centrale Commissie Dierproeven

03 APR 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*	24400
☐ Nee	> *U kunt geen aanvraag doen*	

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

Naam instelling of organisatie	Genmab	
Naam van de portefeuillehouder of diens gemachtigde	[redacted]	
KvK-nummer	30169902	
Straat en huisnummer	Yalelaan	60
Postbus		
Postcode en plaats	3584 CM	Utrecht
IBAN	[redacted]	
Tenaamstelling van het rekeningnummer	[redacted]	

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		
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		
Telefoonnummer	[redacted]	
E-mailadres	[redacted]	

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

2 Over uw aanvraag

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

3 Over uw project

3.1	Wat is de geplande start- en einddatum van het project?	Startdatum	1 - 5 - 2017
		Einddatum	1 - 5 - 2022
3.2	Wat is de titel van het project?	Research and Evaluation of therapeutic test antibodies in tumor models in mice and rats	
3.3	Wat is de titel van de niet-technische samenvatting?	Onderzoek en Evaluatie van therapeutische test antilichamen in kanker modellen in muizen en ratten	
3.4	Wat is de naam van de Dierexperimentencommissie (DEC) aan wie de instellingsvergunninghouder doorgaans haar projecten ter toetsing voorlegt?	Naam DEC	Utrecht
		Postadres	Bureau van de DEC Utrecht Huispostnummer [redacted] Postbus 85500 3508 GA Utrecht
		E-mailadres	dec-utrecht@umcutrecht.nl

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	Utrecht
Datum	30 - 3 - 2017
Handtekening	

Centrale Commissie Dierproeven

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'.	24400
1.2	Provide the name of the licenced establishment.	Genmab
1.3	Provide the title of the project.	Research and Evaluation of therapeutic test antibodies in tumor models in mice and rats

2 Categories

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

- ☐ Basic research
- X 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.

At our company we concentrate on the generation of new recombinant antibodies, and new innovative antibody formats for the treatment of cancer. We continuously invent and generate potential new antibodies and test these pre-clinically as well as evaluate those antibodies in clinical studies.

Cancer is one of the leading causes of death in Western countries including the Netherlands. Many therapies are applied to combat this disease, such as radiation therapy, chemotherapy and immuno-therapy. However, the complexity and heterogeneity of cancer contribute to the fact that not all patients respond to the therapy, or that the cancer returns after a number of years.

The last decade, immunotherapy is an expanding field in the treatment of cancer patients. It is a type of cancer treatment designed to boost the body's natural defenses to fight the cancer. It uses substances either made by the body or in a laboratory to improve or restore immune system function. For example recombinant antibodies for therapeutical use in patients with cancer and/or chronic inflammatory diseases have been proven to be very successful (Scott AM, Nature reviews, 2012).
Recently, the concept of immunotherapy is even appointed as the breakthrough in the treatment of cancer (E Goldin, Nature medicine 2013; J cousin Frankel, Science 2013), indicating the rapid movement of this field.

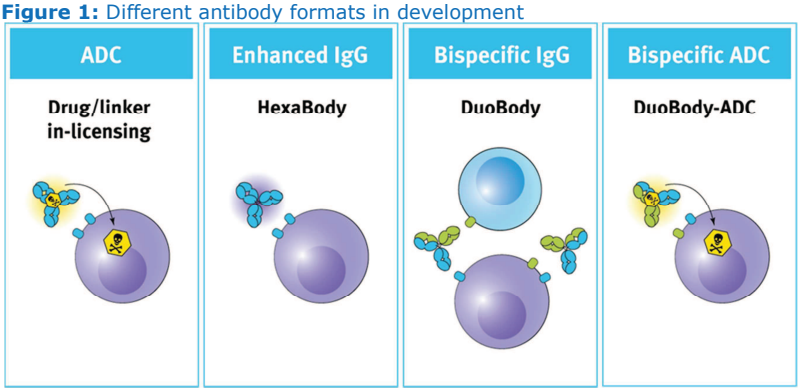
However, because of the heterogeneous characteristics of cancer, still a large number of cancer patients have to face the recurrence of cancer and thus improvement is still required. Though, according to the AACR Cancer progress Report 2016, several new medical products for the treatment of cancer have been approved by the FDA in the last years, it is expected that in the United States in 2016 nearly 600.000 patients will die from some form of cancer (Cancer Statistics 2016, Siegel RL et al, Ca Cancer J Clin, 2016). Therefore, development of new and modified improved antibody formats is a continuous urgent need to further enhance the efficacy and/or safety of these antibody drugs in patients.

At our company we yearly evaluate, in a multidisciplinary team of scientific experts, a large number of novel potential cancer targets that are of interest for antibody targeting therapy. This is done by means of literature search, competitor landscape analysis, collaborations and in vitro studies. Of these targets only a few targets will be selected to start the generation of antibodies for. We have the objective to go forward with only the most promising cancer targets where we see the potential to create new innovative antibody formats for.

The final goal is to generate differentiated antibody therapeutics that are more potent and/or safer than antibodies/drugs currently in the clinic, and have potential to be best-in-class or first-in-class. These criteria will be taken along during the whole antibody selection procedure.
The number of new antibody targets taken into antibody development will differ per year, but will in general be 10-30 per year. This will be done with or without external collaborations.

The antibody formats to be applied to the cancer targets that are designed thus far, and currently in the development by our company are: 'Antibody Drug Conjugates' (ADCs), were a drug/toxin is coupled with a linker to the antibody; Enhanced IgG formats, where enhanced immune effector functions are established; bispecific IgG antibodies, where a dual targeting can be established; and 'bispecific-ADCs', bispecific antibodies with a drug linker (figure 1).

Figure 1: Different antibody formats in development



The following pre-clinical process includes intensive in vitro testing, such as binding characteristics and functional characteristics of the antibody that leads to the kill of the tumor cells or inhibition of tumor cell growth. Besides these initial in vitro processes needed for the first step of antibody characterization, in

vivo efficacy testing in tumor models and pharmacokinetic analysis in mice and rats are inevitable, since only animal studies reflect the complexity as seen in humans/patients.

Recent studies have revealed that new antibody formats can indeed be more potent for the treatment of some cancers compared to (antibody-) drugs already in the clinic. For example, antibody-drug conjugates (ADC), can be more effective in the inhibition of the tumor growth compared to the non-conjugated antibody, as has been demonstrated with Herceptin-DM1 which has been shown to be very effective in breast cancer patients [redacted] Also our own preclinical findings show complete regression of several implanted human tumors, without significant side effects when using ADCs. [redacted] Furthermore, there are strong indications that the use of other new antibody formats developed at our company, such as bispecific antibodies (DuoBody) or HexaBody, can strengthen the potency and specificity of antibodies for the treatment of cancer ([redacted].

The research described in this project application focusses on the selection, research and evaluation of antibodies in tumor models in mice and rats and will be a direct continuation of our current research program ("Development of antibodies for therapeutic use: Research and evaluation on subcutaneous tumor models, optical imaging and imaging of subcutaneous tumor models in rodents). By performing these in vivo experiments we aim to get insight in

- the antibody drug efficacy in a "full body system"
- the mechanism of action of the antibody drug in vivo
- optimal dosing of the antibody drug
- Timing and location of activity of the antibody drug
- The tumor type/indication most suitable for the application of the antibody drugs

To explore this, we require a diversity of specific in vivo tumor models to allow the analysis and evaluation of the different antibody-drugs. The specific models will be set up and designed, based on the antibody format, the molecule and the cancer type that is targeted by the respective antibodies.

Parallel to the studies described in this project application, we are studying related processes which are part of a successful antibody generation, like immunizations in rodents and pharmacokinetic studies. These related processes are described in two different parallel project applications: "Investigation of pharmacokinetics of antibody formats in rodents" and "Immunization of rodents (transgene for human IgG)".

In short: selection, research and evaluation of the efficacy of new antibodies or antibody formats in a diversity of specific in vivo tumor models is very important for the development of therapeutic antibodies and will give us insight into the mechanisms of actions of these drugs. This will help us to translate and predict the potency of these antibody drugs in cancer patients much better and will help us in the design of clinical trials leading to an improved benefit for cancer patients in the future.

3.2 Purpose

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

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

The main objective of the study described in this application is selection, analysis and evaluation of therapeutic antibodies by evaluating anti-tumor efficacy in a diversity of in vivo tumor models in mice and rats.
The need for such studies is elementary for moving the best antibody candidate forward and bringing a new medicine successfully to the clinic as we have done for several antibodies (such as an anti-CD20 antibody (Arzerra®), for the treatment of chronic leucocyte leukemia, and for an anti-CD38 (Darzalex®), for the treatment of multiple myeloma).

3.3 Relevance

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

Evaluation and research of new antibodies drug candidates or new antibody formats in mice or rat tumor models will give insight in the mode of action of the drugs.
Furthermore, by analyzing the different animal tumor models described in this application, we will gain understanding in the use of these different tumor models in antibody therapy and the translatability into cancer patients. Each tumor model type will have its own characteristics, advantages and disadvantages and by studying this (for examples by taking out the tumor and explore the target expression or the immunological cell content) we will gain insight into these models, which will help to refine the design of future tumor models and clinical trials for cancer patients.
Scientific relevant findings will be communicated to collaborators and/or published at specific conferences and/or in scientific magazines.

3.4 Research strategy

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

Before in vivo studies will start, an intensive selection process is performed on a large antibody panel by means of in vitro assays determining the specific target binding and functional activity. Only a small number of the best antibodies will move forward and will be selected for further in vivo research, such as anti-tumor efficacy studies.

Depending on the molecule and the cancer type that the antibody targets and the mechanism of action of the antibody format (ADC, DuoBody, HexaBody, or others) a diversity of specific in vivo tumor models will be designed, set up and evaluated as is described in 3.4.2 and in both appendices animal procedures.

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.

All models described in this project application are animal tumor models.
Based on the general outline of the experimental design of each individual animal experiment, the tumor model described in this application is divided in 2 different animal procedures.

The majority of tumor model experiments [redacted] will be performed as the animal procedure: "subcutaneous tumor models". The remainders of the experiments that will be performed fall under the second animal procedure: "orthotopic tumor and metastasis models".

A short description of the two animal procedures is described below. A detailed description can be found in the two independent appendices of the animal procedures:

1) Animal procedure 1: Subcutaneous tumor models:
   Subcutaneous injection of tumor cells/fragments in mice or rats followed by tumor volume measurements by Caliper and optional with imaging techniques*

2) Animal procedure 2: Orthotopic tumor (injection of tumor cells at the organ of the cell's origin) and metastasis models:

Different ways of tumor cell injection will be applied in this procedure:

a) Orthotopic tumor cell inoculation without surgery:
Injection of tumor cells in easy accessible organs such as in the mammary gland (subcutaneously into the fat pads), or dermis (intradermally)

b) Orthotopic tumor cell inoculation with surgery:
Direct injection of tumor cells in respective organs after opening of the animal's body, (such as pancreas, ovarium, prostate, etc.), or indirect injection (in some incidences) into organs (such as intra-splenic or portal vein injection (imaging* and/or scoring of tumor loci and/or resection and weighing of tumor in the liver).

c) Intravenous tumor cell inoculation: Injection of tumor cells into the tail vein. This injection method does not need surgery or anesthesia.

d) Intracardiac tumor cell inoculation: Injection of tumor cells into the left ventricle of the heart.

e) Intraperitoneal tumor cell inoculation: Injection of tumor cells into the peritoneal cavity.

In this procedure, tumor development will be mainly followed by imaging* and/or alternatively by caliper measurement and/or scoring of tumor loci and/or resection and weighing of tumor.

*Imaging techniques can include: bioluminescence or fluorescence, radioactivity, CT/PET/SPECT in combination with labeled cells or antibodies or tracers/proteins.

In all these animal procedures three different types of models can be selected based on the antibody target and antibody format:

- Xenograft models: in which mostly human tumor cells/fragments will be implanted in mice or rats.
- Syngeneic models: in which the tumor to be injected as cells or fragments, as well as the host are from the same species/strain (mouse or rat)
- Humanized models: In which the immune system of the mouse or rat is "humanized", such as:
  - Engraftment models with human peripheral blood mononuclear cells (PBMCs)
  - CD34+ Hematopoietic Stem cell (HSC) reconstituted humanized
  - Genetically engineered mouse (and rat) models (GEMMS)

Based on the research strategy, antibody target and antibody format, the most optimal model will be selected.

3) Animal procedure 3: Practicing of techniques for Orthotopic tumor and metastasis models:
In case of new models have to be established for the orthotopic tumor and metastasis models that include surgery or intracardiac injection, we expect the need for practicing to optimize the succes rate of the model establishment and antibody efficacy testing in these tumor models.

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.

We make selections of the animal procedures and models described in paragraph 3.4.2 mainly dependent on the antibody target and on the cancer type where the tumor antigen is expressed (see in figure 2).

Fig 2a: [redacted]

Fig 2b: [redacted]

The outcome of the animal tumor model studies will give us insight and knowledge on the activity and mechanism of action of the drug and will be an important step in the decision making process. Based on this, decisions will be made to either progress the antibody drug candidates forward in the development process, or to be put on hold.
Furthermore, these animal tumor model experiments are very important in the clinical trial design with respect to indication/cancer type selection, dosing and the possibility of biomarkers analysis.

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	Subcutaneous tumor models
2	Orthotopic tumor and metastasis models
3	Practising of techniques for orthotopic tumor and metastasis models
4	
5	
6	
7	
8	
9	
10	

Centrale Commissie Dierproeven

3.

Format
Niet-technische samenvatting

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

1 Algemene gegevens

1.1	Titel van het project	Onderzoek en Evaluatie van therapeutische test antilichamen in kanker modellen in muizen en ratten
1.2	Looptijd van het project	5 jaar
1.3	Trefwoorden (maximaal 5)	Antilichamen, kanker, tumor modellen

2 Categorie van het project

2.1 In welke categorie valt het project.

U kunt meerdere mogelijkheden kiezen.

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

3 Projectbeschrijving

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

Kanker is een van de belangrijkste doodsoorzaken in westerse landen waaronder ook in Nederland. Vele therapieën worden toegepast om deze ziekte te behandelen. Echter, de complexiteit en de heterogeniteit van kanker dragen er toe bij dat niet alle patiënten reageren op de huidige therapie, of dat de kanker na een aantal jaren weer terugkeert.
Recombinante antilichamen die gebruikt worden als therapie bij patiënten met kanker (en/of chronische ontstekingsziekten) hebben de laatste jaren bewezen zeer succesvol te zijn. De ontwikkeling van nieuwe en geoptimaliseerde antilichamen blijft echter nodig om de werkzaamheid te verbeteren en veiligheid van deze medicijnen bij patiënten te verhogen.

		Dit project betreft de ontwikkeling van nieuwe verbeterde antilichamen voor gebruik in de behandeling van kanker. In een uitgebreid preklinisch proces worden de beste antilichamen geselecteerd alvorens deze in diermodellen worden getoetst. Het antilichaam met uiteindelijk de beste karakteristieken zal in patiënten getest gaan worden. Het specifieke doel van de experimenten beschreven in dit project is de selectie, analyse en evaluatie van nieuwe verbeterde antilichamen in proefdier modellen voor kanker. Hiertoe worden muizen of ratten ingespoten met kankercellen of kleine stukjes kanker weefsel, waarna groei van deze kankercellen in de muizen of ratten kan plaatsvinden. Door middel van behandeling van de muizen en ratten met de nieuwe verbeterde antilichamen zal geanalyseerd worden of de tumor geheel geëlimineerd kan worden, de tumorgroei geremd kan worden en hoe dit proces bewerkstelligd wordt.
3.2	Welke opbrengsten worden van dit project verwacht en hoe dragen deze bij aan het wetenschappelijke en/of maatschappelijke belang?	Kennis over het werkingsmechanisme van de therapeutische effectiviteit van de nieuwe verbeterde antilichamen is belangrijk voor de voorspelbaarheid van de effectiviteit van deze antilichamen in kanker patiënten. Eveneens zal dit onderzoek inzicht geven in de complexiteit en de heterogeniteit van kanker. Wetenschappelijk interessante bevindingen zullen dan ook in publicaties vastgelegd worden
3.3	Welke diersoorten en geschatte aantallen zullen worden gebruikt?	In totaal verwachten we dat er 20525 dieren gebruikt gaan worden. Daarvan zijn er 18050 muizen en 2475 ratten.
3.4	Wat zijn bij dit project de verwachte negatieve gevolgen voor het welzijn van de proefdieren?	Omdat er tumor cellen of fragmenten van tumoren bij de muizen en ratten worden ingebracht, kan er ten gevolge van de hieraan gerelateerde tumorgroei, ongerief plaatvinden. Daarnaast kan er in proeven waar menselijke immuun cellen aan de muizen worden toegediend ongerief plaatsvinden doordat deze menselijke immuun cellen een reactie kunnen aangaan met de muizen cellen. Dit zal alleen in een klein deel van de dieren kunnen plaatsvinden.
3.5	Hoe worden de dierproeven in het project ingedeeld naar de verwachte ernst?	De procedures hebben over het algemeen een matig ongerief. In gevallen waarbij er geen tumor ontwikkeling plaatsvindt, verwachten we licht ongerief en in sommige gevallen, waar de muizen of ratten het humane eindpunt bereiken, is het ongerief hoger. We verwachten dat in het grootste deel van de experimenten, in 22% van de dieren licht ongerief zal plaatsvinden, in 75% van de dieren matig ongerief en in 3% van de dieren een ernstig ongerief. In de modellen waar herhaaldelijke imaging onder anesthesie of een operatie plaats vindt is het verwachte ongerief: 10% licht, 87 % matig en 3% ernstig. Een zeer klein deel van de dieren specifiek gebruikt worden voor het oefenen van nieuwe technieken hierbij verwachten we een ongerief van 10% licht, 84 matig en 6% ernstig.
3.6	Wat is de bestemming van de dieren na afloop?	De dieren worden geëuthanaseerd ter beëindiging van de proef. Een klein deel van de muizen of ratten kan in leven gehouden worden voor het oefenen van technieken. Na het oefenen worden deze dieren eveneens geëuthanaseerd.

4 Drie V's

4.1	**Vervanging** Geef aan waarom het gebruik van dieren nodig is voor de beschreven doelstelling en waarom proefdiervrije alternatieven niet gebruikt kunnen worden.	Voor de aanvang van de dierproeven worden per project uitgebreide studies gedaan om de bindings-eigenschappen en werkingsmechanismen van de nieuwe verbeterde antilichamen tegen kanker te analyseren. Met laboratorium technieken kan dit goed onderzocht worden en dat maakt een eerste selectie uit een groot antilichaam panel mogelijk. Echter bevindingen met laboratorium technieken alleen, zullen niet een exclusief en volledig inzicht geven in de anti-kanker werkzaamheid van het antilichaam. Daarom zal een klein deel van de geselecteerde antilichamen in dierproeven getest gaan worden. Tumor modellen in dieren reflecteren een complex systeem dat meer vergelijkbaar is met de kanker van de patiënt, met invloeden van factoren zoals bloedtoevoer, het immuunsysteem en de tumor micro-omgeving. Deze afzonderlijke factoren kunnen met behulp van laboratorium technieken onderzocht worden, echter het is tot op heden niet mogelijk om de combinatie van deze factoren te bestuderen. Het testen van de werkzaamheid van antilichamen in een muis of rat tumor model resulteert dan ook in een breder en vollediger overzicht van de werkzaamheid van de geneesmiddelen en draagt bij aan een betere vertaling naar het gebruik van nieuwe en verbeterde antilichamen bij kankerpatiënten.
4.2	**Vermindering** Leg uit hoe kan worden verzekerd dat een zo gering mogelijk aantal dieren wordt gebruikt.	Alle individuele tumor modellen worden zorgvuldig ontworpen, waarbij rekening gehouden wordt met de wetenschappelijke vraagstelling van de dierproef, de voorspelde werkzaamheid van het antilichaam en de variatie van de tumorgroei; homogeen (weinig variatie in tumorgroei) of heterogeen (veel variatie in tumorgroei). Gebaseerd hierop is een berekening uitgevoerd en een richtlijn gemaakt om de meest optimale groepsgrootte te bepalen voor homogene modellen en/of heterogene tumor modellen. Daarnaast is de verblijftijd van het nieuwe verbeterde antilichaam in de bloedcirculatie van belang voor de daadwerkelijke werkzaamheid van het antilichaam ter plaatse van de tumor. Dit wordt in een parallelle projectaanvraag bestudeerd en gebruikt veel minder muizen dan de tumor model experimenten. Met deze gegevens kunnen we goed de verblijftijd van het antilichaam in bloeds- omloop bepalen. Gezamenlijk met gegevens verkregen uit laboratorium onderzoek kunnen wij beter de effectieve dosis in de tumor modellen voorspellen. Dat leidt uiteindelijk tot een gebruik van minder muizen.
4.3	**Verfijning** Verklaar de keuze voor de diersoort(en). Verklaar waarom de gekozen diermodel(len) de meest verfijnde zijn, gelet op de doelstellingen van het project.	Er is veel ervaring met verschillende soorten tumor modellen in muizen en ratten en er is al veel gepubliceerd welke specifieke muis of rat stammen hierbij gebruikt kunnen of moeten worden. Door deze informatie te gebruiken kunnen we de tumor model experimenten zo optimaal mogelijk inrichten. In een groot deel van de proeven zal gekozen worden voor muisstammen die geen intact immuunsysteem hebben, waardoor een directe afstoting van de ingebrachte menselijke tumor cellen voorkomen wordt. Muizen met een intact immuun systeem worden gebruikt als de bijdrage van het immuun systeem van belang is voor de effectiviteit van het nieuwe verbeterde antilichaam. Het gebruik van ratten zal voornamelijk toegepast worden indien er herhaalde bloedafname zal plaatsvinden. Dit kan bijvoorbeeld nodig zijn bij het volgen van stoffen in het bloed die gerelateerd zijn aan de tumor ontwikkeling of de effectiviteit van het antilichaam. De betreffende stoffen zullen door middel van laboratorium technieken geanalyseerd worden.

Vermeld welke algemene maatregelen genomen worden om de negatieve (schadelijke) gevolgen voor het welzijn van de proefdieren zo beperkt mogelijk te houden.	Om de tumorgroei gerelateerde gevolgen zo klein mogelijk te houden vindt er gedurende de hele proef intensieve observatie plaats, die op momenten van verwacht of verhoogt ongerief geïntensiveerd zullen worden. Het tumorvolume wordt minimaal 2 maal per week gemeten. De dieren worden gedood zodra een tumor het maximum toegestane tumorvolume dreigt te bereiken. Zo verminderen we het ongerief van de dieren door een groot tumorvolume. Voor het detecteren van uitzaaiingen of de aanwezigheid van tumoren in inwendige organen gebruiken we beeldvormende technieken. Hierdoor kunnen we ook in deze experimenten anticiperen op het ontstane ongerief door middel van tumorgroei. Met betrekking tot het ontstaan van open wonden op de plaats van de tumoren hebben wij een instructiehandleiding gemaakt hoe te handelen wanneer een open wond wordt waargenomen. Dit helpt bij het schatten van het ongerief, waarbij er beter en sneller beslissingen genomen kunnen worden zodat het ongerief zo kort mogelijk, en de wetenschappelijke waarde van de proef zo hoog mogelijk gehouden kan worden. Tot slot zorgen we ervoor dat het betrokken personeel bekwaam blijft door middel van het volgen van trainingen, door deel te nemen aan cursussen of door het leren van nieuwe technieken.

5 In te vullen door de CCD

Publicatie datum	
Beoordeling achteraf	
Andere opmerkingen	

Centrale Commissie Dierproeven

4.

Appendix
Description animal procedures

1. This appendix should be enclosed with the project proposal for animal procedures.
2. A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
3. For more information, see our website (www.centralecommissiedierproeven.nl).
4. 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'.	24400
1.2	Provide the name of the licenced establishment.	[redacted]
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: 1 Type of animal procedure: 1. Subcutaneous tumor models

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

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

The here described animal procedure includes the **subcutaneous implantation** of human or murine tumor cells mainly, or in some cases patient derived tumor material from approved sources (like CRO's) in:

1. Xenograft models (human tumor cells/fragments in mice or rats),
2. Syngeneic models (murine tumor cells/fragments in mice or rats), or
3. Humanized models (human tumor cells/fragments in mice or rats), mice harboring part of the human immune system (HIS). Examples of HIS mouse are:
   - Subcutaneous co-engraftment with human immune cells (f.e. human PBMCs (Peripheral Blood Mononuclear Cells))
   - intraperitoneal or intravenous engraftment with human immune cells (f.e. PBMCs)
   - $CD34^+$ Hematopoietic stem cell (HSC) reconstitution in young immunocompromised mice. Mice will then be purchased as fully humanized animals.
   - Human Transgenic models (mice will be purchased from appropriate sources)

[redacted]

Secondary parameters may include blood, tissue or tumor analysis. In some models these secondary parameters can be regarded as primary readout parameters, for example in vivo tumor models where circulating blood biomarker analysis is important in relation to the antibody drug, or in tumor models, were the effect of the antibody on the tumor microenvironment is an important mechanism of action. In these experiments tumor tissue collection and analysis will be used as primary readout parameter. But in all these studies tumor volumes will be measured.

In case when new subcutaneous tumor models have to be set up, only the tumor development is addressed with respect to tumor take, growth rate and variation. The purpose of these establishment studies is the determination of the tumor cell inoculation conditions. [redacted] Procedure will be the same as describe below, except for the antibody treatment.

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

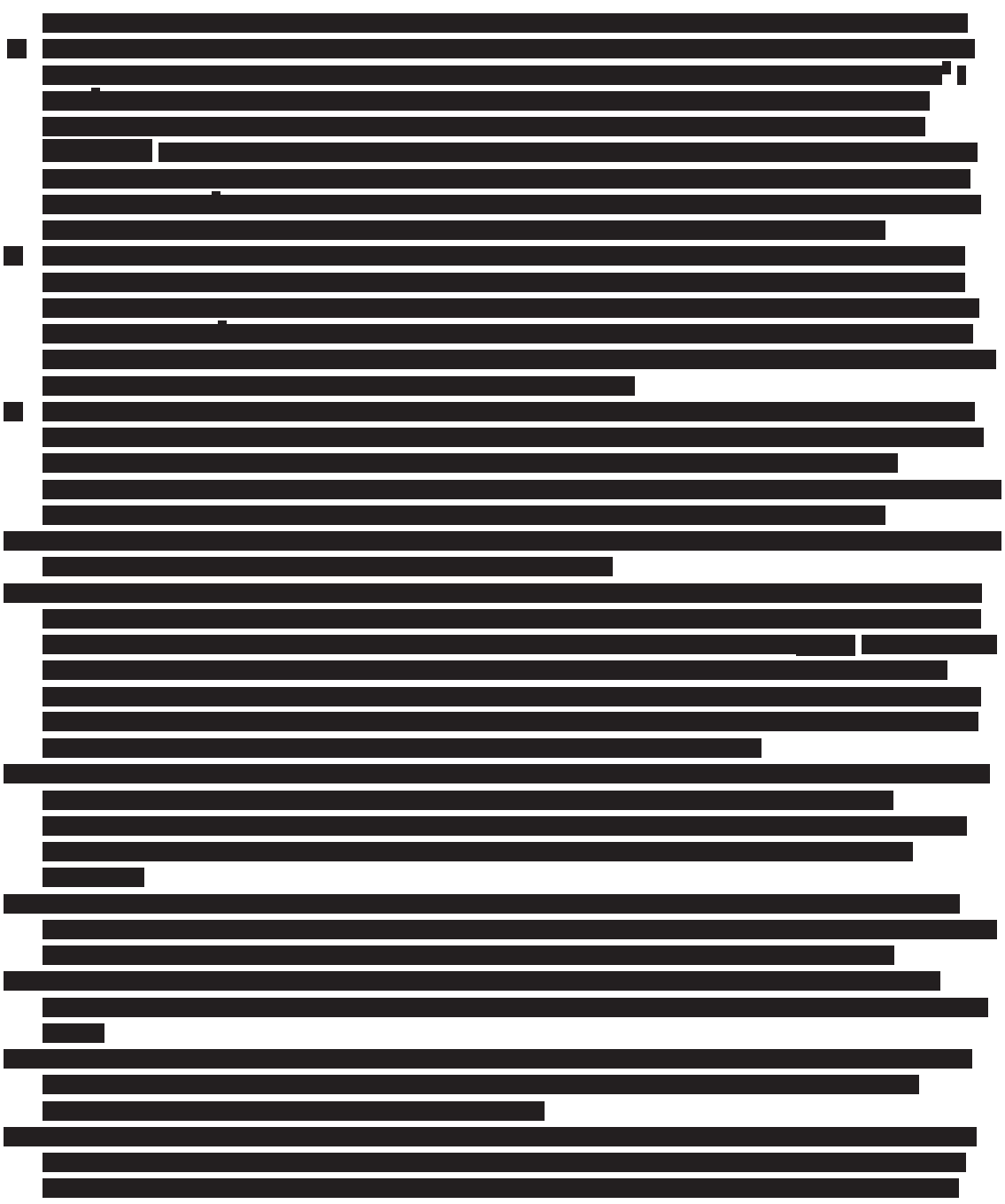
For each animal experiment described in this animal procedure: "subcutaneous tumor models", the following steps will be taken:

- Each individual animal experiment will be discussed, designed and described by a team of experts and will be supported with a rational, study design, group sizes, protocol, statistical analysis and animal welfare expectations.
- All designed animal experiments will be in line with the Code of Practice "Animal experiments in cancer research". Inspectie W&V, (Netherlands Inspectorate for Health Protection, Commodities and Veterinary Public Health), 1999, Zutphen
- Approval of each animal experiment needs to be requested and approved by the AWB and the animal facility.

The general procedure for the subcutaneous tumor models will be as follow

- [redacted]

[redacted]



* total blood sampling will not exceed the maximal limit. Methods for blood sampling include; submandibular vein puncture, vena sephena puncture, tail vein puncture or alternative approved/advised methods.

A general experiment will take normally up to 16 weeks. However, depending on the efficacy of the drug, some animals can be studied for long term resistance, development of immune memory. These studies can take maximally up to 52 weeks.

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

To get an indication for the proper group sizes per study a power/sample size analysis has been done based on the tumor volumes, since the research described in this application indicates "tumor volume" as the primary parameter for tumor burden, a continuous variable readout, calculated on the basis of measurements with (digital) calipers in two dimensions.

Power and groups size analysis:

Group size selection:
Based on the power analysis described above we want to select the following group sizes:
1)

[redacted]

In the group size selection, the scientific outcome and the total number of animal is always taken into consideration.

In all models where antibody treatment is applied and tumor volume will be the primary readout, the relative T/C (tumor volumes of treated animals over control animals values) and "tumor growth inhibition" (TGI) values will be calculated.

Statistical analysis

In general and if possible, a standard statistical analysis will be as much as possible applied as follows:

[redacted]

This analysis can be adapted in the future when new insights appear.

In case of only establishing a new subcutaneous tumor model statistical analysis is not applied, since treatment is absent in these studies.

B. The animals

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

We expect to have an average [redacted] antibody projects active per year, for which we perform in total an

average [redacted] experiments per year. This includes establishment and efficacy testing studies in both animals procedures described in animal procedure I: subcutaneous tumor models as well as the animals experiments described in animal procedure II: orthotopic tumor and metastasis models. (see also Addenda overview table 1 animals)

Within this animal procedure I (s.c.tumor models) we expect to perform [redacted] experiments per year [redacted] models for efficacy testing [redacted] new models to be established).
In average we expect to [redacted] animals per efficacy testing experiments for the s.c. tumor models, and [redacted] animals per new model to be established [redacted] animals per year.
Establishment of new subcutaneous tumor models in general requires less animal per experiment (~ [redacted]/experiment) we expect the development of [redacted] models per year. Which is [redacted] animals per year.

This will lead to the final use within this animal procedure I to a total of [redacted] Of this it is anticipated to need [redacted] in the coming 5 years.

There is extensive experience in tumor models in mice and rats which allows translation of the potency of a novel drug to human patients.
For the animal studies described in this application we intend to use different mouse or rat strains

In most of our studies we aim to test antibodies specifically targeting (human) tumor antigens, where human tumor cell lines (or fragments) need to be used for implantation. In order to prevent rejection of the tumor cells, it is necessary to use immune deficient mice and rats. We most frequently use CB17 SCID or nude mice. Both, nude and CB17 SCID mice, lack the T-cells subset allowing a better tumor outgrowth. CB17 SCID mice, in addition, have no B-cells meaning they also have no mouse IgG. For the rats we mostly use athymic nude rats.

Immune competent mice (mostly BALB/c or C57bl/6) will be used for syngeneic tumor models, where it is important to study the tumor microenvironment. At this moment it is well known that the tumor microenvironment is important for the development of the tumor, and by targeting this environment (for example with a specific antibody) it can lead to tumor growth inhibition. In these cases it is important that the antibody used also recognizes the murine targets. Depending on the origin of the murine tumor cell line the right strain for the respective model needs to be chosen.

NOD SCID mice lack T-cells in addition to B-cells, and also functional macrophages, complement components, as well as functional Natural Killer cells. Therefore, these mice are often used to study bispecific antibodies in which one part is directed against the human target on the tumor cell and the other part is focused on the human immune cell. In these mice, human immune cells will be co-transplanted subcutaneously together with human tumor cells. That allows the efficacy testing of a bispecific antibody targeting the human immune cells and the human tumor cells in an in vivo setting as described in (Labrijn et al, PNAS, 2013). For these studies even mice with additional immune deficiencies such as NSG/NOG or BRGS mice can be used as well.

The use of NSG or NOG (NOD-SCID IL-2Rg$^{-/-}$), but also BRGS mice and NOD SCID mice are described for experiments in which human blood cells are intraperitoneally or intravenously implanted. All these mice are immune deficient, lack B-, T- and NK-cells, and have defects in macrophages. When PBMCs are inoculated in these mice, the human T-cells are the most expanding human cell population and can be used for T-cell targeting drugs, which is nicely shown by Bacac M *et.al*. (Clin Can Res, 2016)
The NSG (and the BRGS mice) or derivatives hereof are used for the already mentioned CD34+cell HSC-His model allowing the expansion of a diversity of human immune cells in these mice (Legrand, PNAS, 2011). These Human Immune System (HIS)-mice will be used in experiments were the tumor microenvironment needs to be studied or when a mechanism of action of the drug includes activation or inhibition of the human immune cells or the tumor microenvironment (Morton et al, Cancer Res 2016).

Rat xenograft models using athymic nude rats are mostly used for experiments (e.g. in biomarker

studies) when repetitive blood samples are required in amounts impossible to get from single mice. Athymic nude rats do not have T cells and allows the outgrowth of implanted human tumor cells, although in many cases a pre-treatment with cyclophoshamide or irradiation might be needed for a proper tumor outgrowth with an acceptable variation. In addition, for the evaluation of novel antibody formats rat models might be of interest because the compliment system in rats allows a better ex vivo analysis since the complement components seem much more stable.

We have a strong preference for the use of female mice, because it has been shown that male mice have a different activity in the innate complement system than female mice (Beurskens F et al, Clin Exp Biol, 1999) and this may influence the effectiveness of the antibodies. To minimize the variation within a single test and between different tests (intra- and inter-variation) we prefer to be limited to one gender. This allows a more accurate statistical analysis and can lead to the use of fewer mice per group. In addition, we prefer females because these are easier to accommodate (less mutual aggression) than males and are easier to regroup. We want to avoid to house animals alone in one cage because of aggressiveness, which is often the case with males.
However, we do include in some experiments a mixture of males and females, for example the HIS mice are both male and female since these animals are seen as individuals and will be analyzed on a more individual basis. Furthermore, sometimes we have experiments with males only, for example when we want to study the efficacy of our antibodies in prostate cancer.

In general we use mice at an age between 5 and 14 weeks old. Young mice normally have a much better tumor take rate. Per experiment we try to keep the mice in an age difference of maximal 2 weeks, since a higher difference might lead to difference in tumor outgrowth. However, sometimes we are restricted to a certain age of the mice because of technical feasibilities. For example humanized NSG or BRGS mice are somewhat older before tumor cell injection, since first the human immune system has to be established, which can take up to 12-14 weeks.

For each animal experiment it will be carefully considered which background, strain, gender and age is required for having an optimal or most translatable readout for the research question to be addressed in that particular experiment and the efficacy of an antibody drug.

In the future other or new mice and rat strains might be developed that allow better engraftment of tumor cells and/or humane immune cells, and might be used in the animal studies as well.

C. Re-use

Will the animals be re-used?

X No, continue with question D.

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

In general the mice/rats will not be re-used.
However in some experiments we may consider using animals for training purposes, such as for practicing new techniques, for re-challenge experiments, or for resistance studies is which a second round of tumor cell injection and/or treatment will be performed. We want to restrict the re-use only to mice/ rats in which the discomfort in the previous study was mild or moderate and when the animal's general state of health and well-being has been fully restored after the previous study.

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

xNo

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

D. Replacement, reduction, refinement

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

Replacement,
In vitro studies to analyze the binding properties of the antibodies to the tumor target and to test their functional anti-tumor activity will always be performed before the start of any in vivo experiment. This allows a first scaling down step of the antibody panel. However in vitro data only will not give an exclusive and complete insight into the anti-tumor efficacy of the antibody of interest. Testing the efficacy of antibodies in in vivo tumor models represents a more complex system which is more comparable to the tumor of the patient and allows the influence of additional factors, such as blood supply, the tumor microenvironment and the contribution of the immune system to the efficacy of the antibody drug. This results in a better and more complete overview on the efficacy of the drugs and allows a better translation to the use in cancer patients.

Reduction,
All the individual tumor models are carefully designed. The variation of the tumor growth (homogeneous or heterogeneous), the predicted efficacy of the drug, and the research question is always taken into account. At each experiment a well thought experimental design is made in which a good scientific outcome is balanced to the total number of mice used in the experiment. This is supported by a power analysis and suggested group sizes described in 2A.
Furthermore bio-distribution of the antibody of interest to the site of the tumor is also very important for the final efficacy of the drug and can only be determined in in vivo settings. To support this feature the pharmacokinetics of the antibody drugs is studied using a parallel project application which in general uses less mice then a tumor model experiment. Using these data we can detect very well the half-life of the specific antibody in circulation. And together with in vitro data we can better predict the effective dose in the tumor models described in this application. Overall following this strategy, this will lead to the use of fewer mice.

Furthermore, we are intensively involved in setting up alternative model systems such as [redacted] In the coming years new techniques will be developed and evaluated if these techniques can successfully replace, refine or reduce the use of animal experiments. However, to explore this, in the first coming years we expect the need of several animal tumor models tumor to evaluate the applicability of the alternative model systems

Refinement
We put a lot of effort in the refinement of the animal tumor models. By having dedicated people we observe, learn and adjust in consecutive studies, so that we know what to expect and in cases of (unexpected) discomfort we can determine how to act to prevent the animals from reaching the "Human End Point" (HEP).
For the maximal tumor size in mice the code of practise "animal experiments in cancer research" (Inspectie W&V, Zutphen/The Hague 1999) describes a tumor volume of 2000 mm3. In our studies we intend to euthanize the mice when a tumor volume above 1500 mm3 is reached. In this way we reduce the discomfort of the mice due to a large tumor size. Also in case of tumor growth in rats, we will euthanize as much as possible the rats before the suggested HEP of 40 cm3 or a diameter of 4.2 cm in the code of practise.

With respect to the appearance of ulcerations at the site of the tumors our team has made an instruction guide how to act and how to handle in case an open wound is observed. This has been discussed extensively within our Animal Welfare Body and is communicated and instructed to the responsible animal caretakers as well. See Addendum III " tumorgroei gerelateerde verschijnselen")

Finally, we make sure that our dedicated personnel remains skilled and will, therefore, be trained regularly, by participating in courses or to learn new techniques in house. Most handlings described in the project applications are standard techniques, and in cases when new techniques need to be learned we prefer to first practise on dead or terminal animals under anaesthesia.

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

The following measures will be taken to minimize the tumor model related discomfort

1) Intensive observation for clinical signs (like abnormal behaviour, posture and appearance) will be done 1-2 times a week, and increased to 3 times a week in periods were we can expect discomfort.
2) 1 to 3 times a week tumor volumes will be measured and when tumors are above 1500 mm3 the mice will be euthanized, to prevent as much as possible that animals reach the human endpoint of 2000 mm3.
3) In case of models that show a wound at the location of the tumor, observation is intensified or preliminary stopped before severe ulcerations appear.
4) Body weight measurements are included in all experiments were discomfort is expected or when it is unknown.

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?

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

The pain and discomfort induced by the anaesthesia is of higher discomfort then the tumor cell and antibody injection.

x Yes > Indicate what relieving methods will be used and specify what measures will be taken

to ensure that optimal procedures are used.

- In cases were imaging or a terminal hart puncture is applied, the animals will first receive anaesthesia.

I. Other aspects compromising the welfare of the animals

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

In some humanized mice models which are adjusted with humane immune cells, we can expect "Graft versus Host Disease"

Explain why these effects may emerge.

- In these models human PBMCs are injected i.p. or i.v., the human T-cells are the most expanding human cell population. The cytotoxic T-cells then recognize the mouse as non-self and can react with the murine body cells. This graft versus host reaction can be observed by clinical signs such as abnormal behaviour, posture and appearance and can be expected 4-6 week after i.p. or i.v. injection of human PBMCs.
- When the PBMCs are subcutaneous co-injected with the tumor cells, only in a few cases these clinical signs are observed. And a graft versus host reaction can be recognized occasionally in these incidences as well.
- When $CD34^+$ hematopoietic stem cells are implanted in young mice (of 1 -3 weeks old), the human immune cells will be trained in the thymus to recognize the mouse cells as "selves" and, therefore, no graft versus host reaction (or only at a very minimal level) will appear in these mice

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

In the period of expected graft versus host reaction, observation will be increased and if possible mice will be preventively euthanized before reaching this point.

J. Humane endpoints

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

☐ No > Continue with question K.

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

Since tumor cells/fragments are implanted in the mice/rats, we can expect tumor growth-related adverse effects, which can lead to tumor related humane endpoints. We use the following humane endpoints as guidance:

1. A large tumor volume is a specific human endpoint in these studies and will be minitored by caliper measurements troughout the whole study: According to Code of practise animal experiment in cancer research) HEP tumorvolume = 2000 mm3 for mice, and 40 cm^3 or a max diameter of 4.2 cm in rats. However in most cases we stop the mice when tumorvolumes of > 1500 mm^3 are reached
2. Ulcerations at the site of the tumor, are a specific HEP in these studies.
3. Body weight loss (> 20%)
4. Clinical signs such as abnormal behaviour, dyspnea (diffuculties with breathing), salivation, no response on incentives, apathy, involuntary shaking of the body or limbs (tremor), convulsion (where the body muscles contract and relax rapidly and repeatedly), self-mutilation, piloerection, abnormal non-physiological posture, paralysis, severe Diarrhea. The level of severeness of these clinical signs can be used to determine if the humane HEP is reached.
5. Graft versus Host Disease (see I)

When a HEP is reached animals will be taken out of the study and will be prematurely euthanized in most of the cases. In some cases where we observe in an early time-point of the study an (unexpected) adverse event close to HEP we might consider to leave the animals in the study for a very short time (1-3 days) because a repetition of the entire study would consume again several mice. In this case we will increase the observation rate, put them eventually in a separate warmed cage, and/or, when observing weight loss, give a subcutaneous PBS injection to recover body fluid. If after 3 days the animal still is not recovered, the rat/mice will be euthanized

Indicate the likely incidence.

Since we follow the tumor volume frequently, we can anticipate when the HEP with respect to tumor size will be reached. Therefore, only a minimal number of animals will reach the maximal tumor volume as humane endpoint.
In models in which we have intensive experience we do not expect that any of the animals will reach the humane endpoint. However, in models that have heterogeneous tumor growth and/or where we have less experience we can expect more mice to reach the humane endpoint.

Appearance of ulceration at the site of the subcutaneous located tumor is dependent on the tumor cell type, e.g. some tumor have more mucus production, appear therefore more tumid, and can cause irritation of the skin at the site of the tumor more severely than others. For most tumor models we know in advance that ulceration might occur, and we, therefore, can anticipate on the appearance of ulceration. In addition, at the moment when a wound is observed, the monitoring of the whole experiment will be intensified or when it has no impact on the scientific outcome, animals will be euthanized before a severe ulceration appears.

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').

The procedures have in general a moderate level of discomfort. In some cases were no tumor development takes places we can expect only mild discomfort and in some cases when animals reach unexpectedly the HEP the discomfort is higher. In a typical experiment we expect:
In 22% a mild discomfort
In 75% a moderate discomfort
In 3% a severe discomfort

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.

Animals are specifically bred for this procedure and they cannot be used for another type of 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

1. This appendix should be enclosed with the project proposal for animal procedures.
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3. For more information, see our website (www.centralecommissiedierproeven.nl).
4. 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'.	24400
1.2	Provide the name of the licenced establishment.	[redacted]
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 2 Type of animal procedure: 1. Orthotopic tumor and Metastasis models

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

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

The here described animal procedure includes tumor models with orthotopic implantation and other metastasis models of human or murine tumor cells mainly, or in some cases patient derived tumor material from approved sources (like CROs) in:

1. Xenograft models (human tumor cells/fragments in mice or rats),
2. Syngeneic models (murine tumor cells/fragments in mice or rats), or
3. Humanized models (human tumor cells/fragments in mice or rats), mice harboring part of the human immune system (HIS). Examples of HIS mouse are:
   - Subcutaneous co-engraftment with human immune cells (f.e. human PBMCs (Peripheral Blood Mononuclear Cells))
   - intraperitoneal or intravenous engraftment with human immune cells (f.ePBMCs)
   - $CD34^+$ Hematopoietic stem cell (HSC) reconstitution in young immunocompromised mice. Mice will then be purchased as fully humanized animals.
   - Human Transgenic models (mice will be purchased from appropriate sources)

Within this animal procedure different methods for tumor cell inoculation can be used to allow orthotopic tumor growth or metastasis formation as indicated below from a) to e):

a) Orthotopic tumor cell inoculation without surgery:

Breast cancer cell lines can be implanted subcutaneously into the "fat pads" of mice which represent the mammary glands of mice or rats. For melanoma models the tumor cells can be implanted directly intra-dermally. This organ specific injection route can in some cases lead to metastasis in other organs. These implantation methods do not need a surgery involving anesthesia.

b) Orthotopic tumor cell inoculation with surgery:
Cancer models, like those derived from pancreas, prostate, kidney, ovary, colon, or other solid cancer forms, need a surgery to get access to the organ of interest in order to establish successfully an orthotopic tumor model in mice or rats. This organ specific injection route can in some cases lead to metastasis in other distant organs. These implantation methods do need anesthesia and in most cases pain relieving methods as well.

c) Intravenous tumor cell inoculation:
Injection of tumor cells in the bloodstream can lead to metastasis formation in different organs. For leukemic cells this often leads to homing and metastasis in different organs and is also known as "a disseminated tumor model". When intravenous injection in the tail vail is applied for other, mainly "bigger" solid cancer cell, the tumor cells home into the lungs and can be used as a lung metastasis model. This injection method does not need surgery or anesthesia.

d) Intra-cardiac tumor cell inoculation:
Injection of tumor cells into the left heart ventricle can be applied when metastasis to different organs is of interest and when metastasis mainly into the lungs needs to be avoided. This method does not include surgery, but intra-cardiac injection needs the use of anesthesia.

e) Intraperitoneal tumor cell inoculation:
This method can be selected when the influence of the peritoneal microenvironment needs to be studied. In that case for example gastric cancer cells or alternative cell will be implanted intraperitoneally. This injection method does not need surgery or anesthesia.

In general, after tumor cell inoculation, the antibodies of interest (antibody variants, - formats, Antibody Drug Conjugates (ADCs), etc. or combinations hereof) will be administrated with or without a combination of cytostatics or other anti-cancer agents in these models. Tumor growth (in mm3) will be followed in time by imaging (bioluminescence/fluorescence) or alternatively by Caliper measurements, or a combination hereof. "Tumor volume" measured by imaging or by caliper will be the primary readout parameter. Differences in tumor volume, growth rate, tumor growth inhibition or tumor regression induced by the antibody variants compared to the control group and to each other will be addressed in these studies. The outcome will be supported by statistical analysis, where possible, as indicated below.

Secondary parameters may include blood, tissue or tumor analysis. In some models these secondary parameters can be regarded as primary readout parameters. For example in vivo tumor models where circulating blood biomarker analysis is important in relation to the antibody drug. Or in tumor models, were the effect of the antibody on the tumor microenvironment is an important mechanism of action. In these experiments tumor tissue collection and analysis will be used as primary readout parameter. But in all studies tumor volumes will be measured.

In case new orthotopic or metastasis tumor models have to be set up, only the tumor development is addressed with respect to tumor take, growth rate and variation. The purpose of these establishment studies is the determination of the tumor cell inoculation conditions. [redacted] Procedure will be the same as describe below, except for the antibody treatment.

When more complex orthotopic or metastasis models have to be established, such as tumor cell inoculation procedures with surgery or by intracardiac inoculation, we expect the need for practicing upfront establishment of the specific tumor model. This is described in appendix 3.

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

For each animal experiment described in this animal procedure: "orthotopic tumor and metastasis models", the following steps will be taken:

- Each individual animal experiment will be discussed, designed and described by a team of experts. Supported with a rational, study design, group sizes, protocol, statistical analysis and animal welfare expectations.
- All designed animal experiments will be in line with the Code of Practice "Animal experiments in cancer research". Inspectie W&V, (Netherlands Inspectorate for Health Protection, Commodities and Veterinary Public Health), 1999, Zuthpen
- Approval of each animal experiment needs to be requested and approved by the AWB and the animal facility.

The general procedure for orthotopic tumor and metastasis models will be as follow:

[redacted]

- [redacted]

[redacted]

[redacted]

* total blood sampling will not exceed the maximal limit. Methods for blood sampling include; submandibular vein puncture, vena sephena puncture, tail vein puncture or alternative approved/advised methods

A general experiment will take normally up to 16 weeks. However, depending on the efficacy of the drug, some animals can be studied for long term resistance, development of immune memory. These studies can take maximally up to 52 weeks.

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

To get an indication for the proper group sizes per study a power/sample size analysis has been done based on the tumor volumes, since the research described in this application indicates "tumor volume" as the primary parameter for tumor burden, a continuous variable readout, calculated on the basis of measurements with (digital) calipers in two dimensions.

Power and groups size analysis:

[redacted]

[redacted]

Group size selection:
Based on the power analysis described above we want to select the following group sizes:

[redacted]

[redacted]

Statistical analysis

[redacted]

This analysis can be adapted in the future when new insights appear.

In case of only establishing a new orthotopic or metastasis tumor model statistical analysis is not applied, since treatment is absent in these studies

B. The animals

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

We expect to have an average of [redacted] antibody projects active per year, for which we perform in total an average of [redacted] experiments per year. This includes both animals procedures described in animal procedure I: subcutaneous tumor models as well as the animals experiments described in animal procedure II: orthotopic tumor and metastasis models. (see also Addenda Table 1: number of animals)

Within this animal procedure II (orthotopic tumor and metastasis models) we expect to perform [redacted] experiments per year. [redacted] models for efficacy testing and [redacted] models to be established).

In average we expect to use [redacted] animals per experiments for the orthotopic tumor and metastasis models, which will be [redacted] animals per year.

For the establishment of new orthotopic or metastasis tumor models we expect the use of [redacted] animal per experiment. We expect the development of [redacted] models per year. Which is [redacted] animals per year.

This will lead to the final use within this animal procedure to a total of [redacted] animals in 5 years. Of this it is anticipated to need [redacted] the coming 5 years.

In addition we expect to need of 25 newly ordered animals per year for practising of the different techniques described in this project application, which will be [redacted] animals for practicing purposes only for the coming 5 years. We expect the need of [redacted] in total for practicing purposes. This is described in appendix 3: practicing of techniques for orthotopic and metastasis models.

There is extensive experience in tumor models in mice and rats which allows translation of the potency of a novel drug to human patients.
For the animal studies described in this application we intend to use different mouse or rat strains

Especially for orthotopic and metastasis models and/or where imaging is applied we will use mouse and rat strains recommended in the literature for a particular orthotopic/metastasis model, or will use animal strains we have experience with (see below). For syngeneic models we have to use the strains where the cells originated from.

In most of our studies we aim to test antibodies specifically targeting (human) tumor antigens, where human tumor cell lines (or fragments) need to be used for implantation. In order to prevent rejection of the tumor cells, it is necessary to use immune deficient mice and rats. We most frequently use CB17 SCID or nude mice. Both, nude and CB17 SCID mice, lack the T-cells subset allowing a better tumor outgrowth. CB17 SCID mice, in addition, have no B-cells meaning they also have no mouse IgG. For the rats we mostly use A-thymic nude rats.

Immune competent mice (mostly BALB/c or C57bl/6) will be used for syngeneic tumor models, where it is important to study the tumor microenvironment. At this moment it is well known that the tumor microenvironment is important for the development of the tumor, and by targeting this environment (for example with a specific antibody) it can lead to tumor growth inhibition. In these cases it is important that the antibody used also recognizes the murine targets. Depending on the origin of the murine tumor cell line the right strain for the respective model needs to be chosen.

NOD SCID mice lack T-cells in addition to B-cells, and also functional macrophages, complement components, as well as functional Natural Killer cells. Therefore, these mice are often used to study bispecific antibodies in which one part is directed against the human target on the tumor cell and the other part is focused on the human immune cell. In these mice, human immune cells will be co-transplanted together with human tumor cells. That allows the efficacy testing of a bispecific antibody targeting the human immune cells and the human tumor cells in an in vivo setting as described in (Labrijn et al, PNAS, 2013). For these studies even mice with additional immune deficiencies such as NSG/NOG or BRGS mice can be used as well.

The use of NSG or NOG (NOD-SCID IL-2Rg$^{-/-}$), but also BRGS mice and NOD SCID mice are described for experiments in which human blood cells are intraperitoneally or intravenously implanted. All these mice are immune deficient, lack B-, T- and NK-cells, and have defects in macrophages. When PBMCs are inoculated in these mice, the human T-cells are the most expanding human cell population and can be used for T-cell targeting drugs, which is nicely shown by Bacac M *et.al*. (Clin Can Res, 2016)
The NSG (and the BRGS mice) or derivatives hereof are used for the already mentioned CD34+cell HSC-His model allowing the expansion of a diversity of human immune cells in these mice [Legrand, PNAS, 2011). These human immune system (HIS)-mice will be used in experiments were the tumor microenvironment needs to be studied or when a mechanism of action of the drug includes activation or inhibition of the human immune cells or the tumor microenvironment (Morton et al, Cancer Res 2016).

Rat xenograft models using athymic nude rats are mostly used for experiments (e.g. in biomarker studies) when repetitive blood samples are required in amounts impossible to get from single mice. Athymic nude rats do not have T cells and allows the outgrowth of implanted human tumor cells, although in many cases a pre-treatment with cyclophoshamide or irradiation might be needed for a proper tumor outgrowth with an acceptable variation. In addition, for the evaluation of novel antibody formats rat models might be of interest because the compliment system in rats allows a better ex vivo analysis since the complement components seem much more stable.

We have a strong preference for the use of female mice, because it has been shown that male mice have a different activity in the innate complement system than female mice (Beurskens F et al, Clin Exp Biol, 1999) and this may influence the effectiveness of the antibodies. To minimize the variation within a single test and between different tests (intra- and inter-variation) we prefer to be limited to one gender. This allows a more accurate statistical analysis and can lead to the use of fewer mice per group. In

addition, we prefer females because these are easier to accommodate (less mutual aggression) than males and are easier to regroup. We want to avoid to house animals alone in one cage because of aggressiveness, which is often the case with males.
However, we do include in some experiments a mixture of males and females, for example the HIS mice are both male and female since these animals are seen as individuals and will be analyzed on a more individual basis. Furthermore, sometimes we have experiments with males only, for example when we want to study the efficacy of our antibodies in prostate cancer.

In general we use mice at an age between 5 and 14 weeks old. Young mice normally have a much better tumor take rate. Per experiment we try to keep the mice in an age difference of maximal 2 weeks, since a higher difference might lead to difference in tumor outgrowth. However, sometimes we are restricted to a certain age of the mice because of technical feasibilities. For example humanized NSG or BRGS mice are somewhat older before tumor cell injection, since first the human immune system has to be established, which can take up to 12-14 weeks.

For each animal experiment it will be carefully considered which background, strain, gender and age is required for having an optimal or most translatable readout for the research question to be addressed in that particular experiment and the efficacy of an antibody drug.

In the future other or new mice and rat strains might be developed that allow better engraftment of tumor cells and/or humane immune cells, and might be used in the animal studies as well.

C. Re-use

Will the animals be re-used?

X No, continue with question D.

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

In general the mice/rats will not be re-used.
However in some experiments we may consider using animals for training purposes, such as for practicing new techniques, for re-challenge experiments, or for resistance studies is which a second round of tumor cell injection and/or treatment will be performed. We want to restrict the re-use only to mice/ rats in which the discomfort in the previous study was mild or moderate and when the animal's general state of health and well-being has been fully restored after the previous study.

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

xNo

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

D. Replacement, reduction, refinement

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

Replacement,
In vitro studies to analyze the binding properties of the antibodies to the tumor target and to test their functional anti-tumor activity will always be performed before the start of any in vivo experiment. This allows a first scaling down step of the antibody panel. However in vitro data only will not give an exclusive and complete insight into the anti-tumor efficacy of the antibody of interest. Testing the efficacy of antibodies in in vivo tumor models represents a more complex system which is more comparable to the tumor of the patient and allows the influence of additional factors, such as blood supply, the tumor microenvironment and the contribution of the immune system to the efficacy of the antibody drug. This results in a better and more complete overview on the efficacy of the drugs and allows a better translation to the use in cancer patients.

Reduction,
All the individual tumor models are carefully designed. The variation of the tumor growth (homogeneous

or heterogeneous), the predicted efficacy of the drug, and the research question is always taken into account. At each experiment a well thought experimental design is made in which a good scientific outcome is balanced to the total number of mice used in the experiment. This is supported by a power analysis and suggested group sizes described in 2A.
Furthermore bio-distribution of the antibody of interest to the site of the tumor is also very important for the final efficacy of the drug and can only be determined in in vivo settings. To support this feature the pharmacokinetics of the antibody drugs is studied using a parallel project application which in general uses less mice then a tumor model experiment. Using these data we can detect very well the half-life of the specific antibody in circulation. And together with in vitro data we can better predict the effective dose in the tumor models described in this application. Overall following this strategy, this will lead to the use of fewer mice.

Furthermore, we are intensively involved in setting up alternative model systems such as [redacted] In the coming years new techniques will be developed and evaluated if these techniques can successfully replace, refine or reduce the use of animal experiments. However to explore this, in the first coming years we expect the need of several animal tumor models tumor to evaluate the applicability of the alternative model systems

Refinement
We put a lot of effort in the refinement of the animal tumor models. By having dedicated people we observe, learn and adjust in consecutive studies, so that we know what to expect and in cases of (unexpected) discomfort we can determine how to act to prevent the animals from reaching the "Human End Point" (HEP).
For the maximal tumor size in mice the code of practise "animal experiments in cancer research" (Inspectie W&V, Zutphen/The Hague 1999) describes a tumor volume of 2000 mm3. In our studies we intend to euthanize the mice when a tumor volume above 1500 mm3 is reached. In this way we reduce the discomfort of the mice due to a large tumor size. Also in case of tumor growth in rats, we will euthanize as much as possible the rats before the suggested HEP of 40 cm3 or a diameter of 4.2 cm in the code of practise.

In case of orthotopic tumors or metastasis, we will follow in most cases the tumor development by imaging, and can anticipate on the appearance of HEP related with tumor growth and act accordingly to avoid the HEP as much as possible. When performing consecutive studies with orthotopic models we get a feeling on the time lines and the in vivo luciferase activity. We are then even more cautious when animals reach human endpoints.

With respect to the appearance of ulcerations at the site of the tumors our team has made an instruction guide how to act and how to handle in case an open wound is observed. This has been discussed extensively within our Animal Welfare Body and is communicated and instructed to the responsible animal caretakers as well. See Addendum III " tumorgroei gerelateerde verschijnselen")

Finally, we make sure that our dedicated personnel remains skilled and will, therefore, be trained regularly, by participating in courses or to learn new techniques in house. Most handlings described in the project applications are standard techniques, and in cases when new techniques need to be learned we prefer to first practise on dead or terminal animals under anaesthesia. In some cases as additional mice/or rats will be ordered for the use of practising only (as described in Appendix 3).

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

The following measures will be taken to minimize the tumor model related discomfort:

1) Intensive observation for clinical signs (like abnormal behaviour, posture and appearance) will be done 1-2 times a week, and increased to 3 times a week in periods were we can expect discomfort.
2) 1 to 3 times a week tumor volumes will be measured and when tumors are above 1500 mm3 tumors will be stopped, so that only a minimal number of mice reach the human endpoint of

2000 mm3. When imaging is applied to follow tumor growth we can (based on imaging values, time point in the study and tumor type) anticipate when HEP can be reached. We will act accordingly to minimize the discomfort of the mice and rats as much as possible, with keeping the scientific value as high as possible.

3) In case of models that show a wound at the location of the tumor, observation is intensified or preliminary stopped before severe ulcerations appear
4) Body weight measurements are included in all experiments were discomfort is expected or when it is unknown.

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?

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

For all treatments with antibodies or other test compounds as well as for the implantation into the mammary fat pads (breast cancer models) or intradermally (melanoma models) or intraveneously (leukemic and other metastasis models) no anesthesia or analgesia is required because the distress for the animals is very low.

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

-For all surgeries as well as intracardiac tumor cell injections the animals will receive anesthesia
-To prevent pain, an analgesic is applied 30 minutes-1 hour before the surgery and can be repeated 24

hours after the implantation.
-The painkillers needed for more severe pain, will only be applied after consultation of the veterinarian

I. Other aspects compromising the welfare of the animals

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

In some humanized mice models which are adjusted with humane immune cells, we can expect "Graft versus Host Disease"

Explain why these effects may emerge.

- In these models human PBMCs are injected i.p. or i.v., the human T-cells are the most expanding human cell population. The cytotoxic T-cells then recognize the mouse as non-self and can react with the murine body cells. This graft versus host reaction can be observed by clinical signs such as abnormal behaviour, posture and appearance and can be expected 4-6 week after i.p. or i.v. injection of human PBMCs.
- When the PBMCs are subcutaneous co-injected with the tumor cells, only in a few cases these clinical signs are observed. And a graft versus host reaction can be recognized occasionally in these incidences as well.
- When $CD34^+$ hematopoietic stem cells are implanted in in young mice (of 1 -3 weeks old),, the human immune cells will be trained in the thymus to recognize the mouse cells as "selves" and, therefore, no graft versus host reaction (or only at a very minimal level) will appear in these mice

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

In the period of expected graft versus host reaction, observation will be increased and if possible mice will be preventively euthanized before reaching this point.

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.

Since tumor cells/fragments are implanted in the mice/rats, we can expect tumor growth related adverse effects, which can lead to tumor related human endpoints. We use the following human endpoints as guidance:

1. A large (visible) tumor volume is a specific human endpoint in these studies and will be minitored by caliper measurements for the breast cancer and melanoma models troughout the whole study: According to Code of practise animal experiment in cancer research) HEP tumor volume = 2000 mm^3 for mice and 40 cm^3 or a max diameter of 4.2 cm in rats. However in most cases we stop the mice when tumorvolumes of > 1500 mm^3 are reached
2. When tumors are not visible, imaging is applied in most studies to follow tumor growth. Based on imaging values, time point in the study and tumor type, we anticipate when the HEP can be reached. Human endpoint is for these non-visible tumors mostly observed by the clinical signs and body weight loss.
3. Ulcerations at the site of the tumor in breast cancer and melanoma orthotopic models have to be considered as a specific HEP in these studies.
4. Body weight loss (> 20%)
5. Clinical signs, most important for orthotopical models since mostly no primary tumor is visible, are: abnormal behaviour, dyspnea (diffuclties with breathing), salivation, no response on incentives, apathy, involuntary shaking of the body or limbs (tremor), convulsion (where the body muscles contract and relax rapidly and repeatedly), self-mutilation, piloerection, abnormal non-physiological posture, paralysis, severe Diarrhea. The level of severeness of these clinical signs can be used to determine if human HEP is reached.
6. For some specific leukemic/metastasis models hind leg paralysis is described as a clinical observation. The tumor cells home to different organs/tissues. The paralysis of the hind legs just prior to death is associated with the presence of neoplastic nodules within the spinal canal. (Jin-Song Yan, et al, Chin J of Cancer 2009) and will be considered as a HEP in these studies.
7. Graft versus Host disease. (this will be only expected in humanized mice, as described in I)

When a HEP is reached animals will be taken out of the study and prematurely euthanized in most of the cases. In some cases where we observe in an early time-point of the study an (unexpected) adverse event close to HEP we might consider to leave the animals in the study for a very short time (1-3 days) because a repetition of the entire study would consume again several mice. In this case we will increase the observation rate, put them eventually in a separate warmed cage, and/or, when observing weight loss, give a subcutaneous PBS injection to recover body fluid. If after 3 days the animals still is not recovered, the rat/mice has to be euthanized.

Indicate the likely incidence.

Especially for the orthotopic models where we can apply caliper measurements (breast cancer and melanoma models), we can anticipate when the HEP with respect to tumor size will be reached. Therefore, only a minimal of animals will reach the max tumor volume as human endpoint.
For orthotopic and metastasis models, where no primary tumor is easily visible, we visualize primary tumors as well as metastases by applying imaging techniques. In that respect we get a quantitative readout for the tumor and metastasis burden. This, but also the timing in the model and the appearance of clinical signs have to be taken into account to address animal's discomfort and HEP.
In models in which we have intensive experience we do not expect that any of the animals will reach the human endpoint. However, in models that have heterogeneous tumor growth and/or where we have less experience we can expect more mice to reach the human endpoint.

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').

The procedures have in general a moderate level of discomfort. In some cases were no tumor development takes plays we can expect only mild discomfort and in some cases when animals reach unexpectedly the HEP the discomfort is higher. In a typical experiment we expect:
In 10% a mild discomfort
In 87% a moderate discomfort
In 3% a severe discomfort

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.

Animals are specifically bred for this procedure and they cannot be used for another type of 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

1. This appendix should be enclosed with the project proposal for animal procedures.
2. A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
3. For more information, see our website (www.centralecommissiedierproeven.nl).
4. 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'.	24400
1.2	Provide the name of the licenced establishment.	[redacted]
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 3 Type of animal procedure: 1. Practicing of several techniques for orthotopic tumor and metastasis tumor models

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

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

The here described animal procedures include only the practicing of different techniques needed to establish orthotopic and/or metastasis models.

We expect the need of additional mice and/or rats for practicing of two procedures described in appendix 2 (orthotopic/metastasis models). These methods include method b) orthotopic tumor cell inoculation with surgery and method d) intracardiac inoculation of tumor cells.
Since we have limited experience with both these techniques, it is important to validate the correct way of tumor cell injection and surgery before establishment and performing antibody efficacy studies. To practice these procedures we will apply standardized techniques that are described in literature or we will be trained by people having experience in the establishment of these models.

Additional animals for practicing are not needed for the following methods: a) orthotopic implantation without surgery, c) intravenous inoculation and e) intraperitoneal inoculation (described in Appendix 2). These techniques are already established and intensively applied in the efficacy testing of the antibodies in development, so no additional practicing is needed. Also for the methods described in appendix 1 (subcutaneous implantation of tumor cells) additional mice /or rats for practicing are not required since this technique is applied in most of our experiments and personnel is already trained and skilled in these techniques.

The parameters in these practising sessions are the successful conduct of proper tumor cell injection techniques in the different organs after surgery (prostate, pancreas, ovarian etc.) or intra-cardially. In addition it is important to monitor the recovery of the animal from the surgery/tumor cell inoculation under anaesthesia and to monitor successful tumor growth.

Typically in training sessions only tumor cells will be inoculated and no antibodies. However, not the tumor take rate or tumor volume will be a primary endpoint but rather the clinical conditions of the mice/rat a few days after the practiced technique/surgery, or tumor cell inoculation. If successfully recovered from surgery, validation of outgrowth of the tumor within the intended organ(s) will be evaluated and imaging of tumor volume can be applied in some of these mice or rats.

In most cases animals will be euthanized a few days after training the tumor cell inoculation. However in cases when animals recover successfully after tumor cell inoculation, it can be considered to follow tumor growth and body weight measurements. This has only the purpose to validate the implantation technique.

These studies do not need the support of statistical analysis.

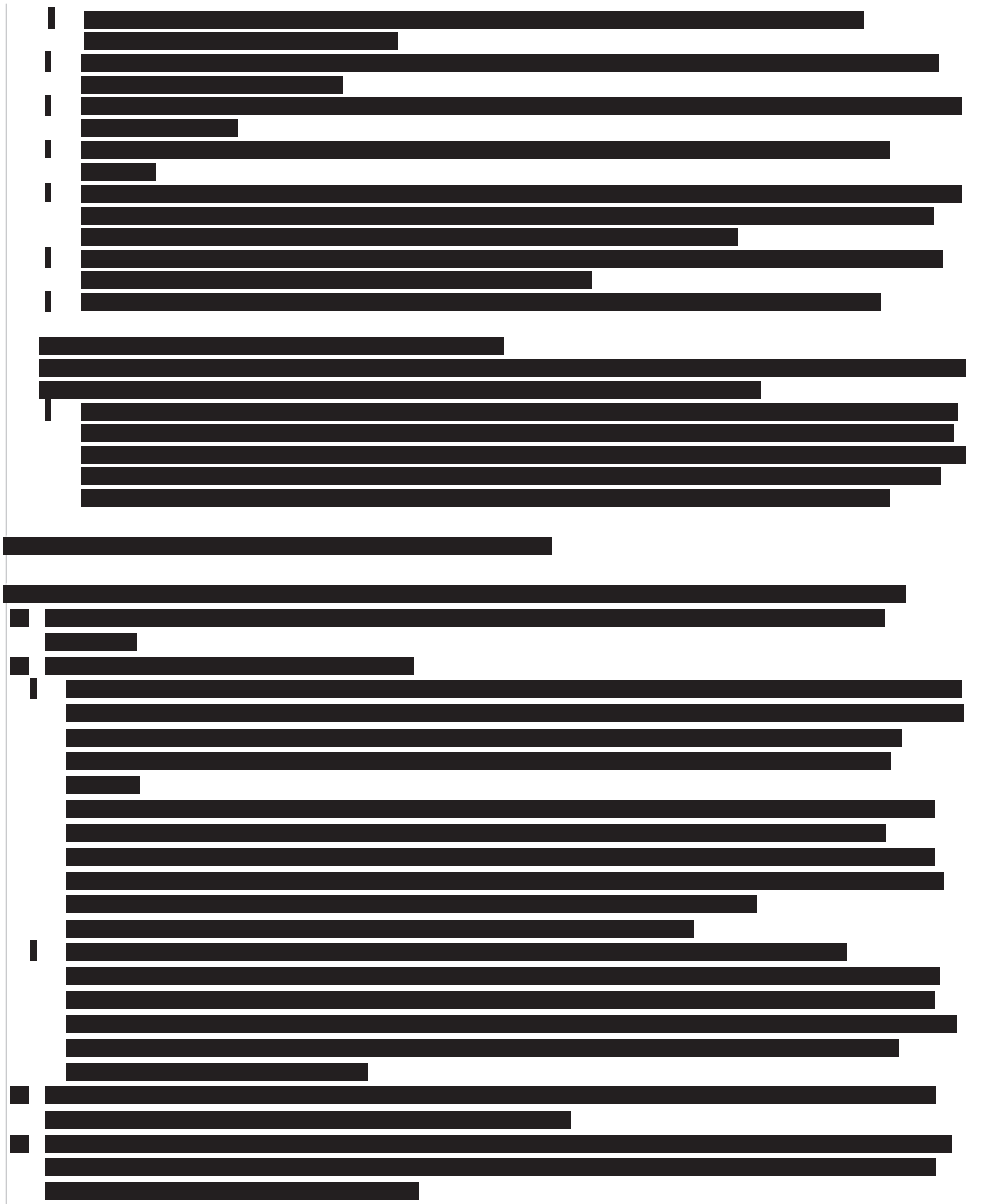
Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Practicing the different techniques described in this animal procedure: "Practising of several techniques for orthotopic tumor and metastasis models" will be comparable to the technique b) and d) as described in Appendix II.
Before initiation of each practicing session, the following steps will be and taken:

- The animal experiment will be discussed, designed and described by a team of experts. Supported with a rational, protocol, and animal welfare expectations.
- All designed animal experiments will be in line with the Code of Practice "Animal experiments in cancer research". Inspectie W&V, (Netherlands Inspectorate for Health Protection, Commodities and Veterinary Public Health), 1999, Zuthpen
- Approval of each animal experiment needs to be requested and approved by the AWB and the animal facility.

The general procedure for practicing of techniques for orthotopic tumor and metastasis models will be as follow:

[redacted]



* total blood sampling will not exceed the maximal limit. Methods for blood sampling include; submandibular vein puncture, vena sephena puncture, tail vein puncture or alternative approved/advised methods

A general practicing procedure will take up to 1 week.
However, in case of successful recovery of the animal after surgery it can be considered to include the steps as described above to monitor the tumor outgrowth. This will allow us to check the specific sites/organs of tumor outgrowth and allow us to validate if the implantation techniques were properly performed. Depending on the tumor growth these can take up to a maximum of 16 weeks.

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

The animals used in this appendix are only used for practicing purposes, and no statistical analysis will be needed to support the primary endpoints of these practicing studies.)

B. The animals

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

The number of animals needed in these practising procedures will be limited to the number of personnel to be trained[redacted] Basic trainings steps (such as localization of the specific organs, orientation and route of injection will be first trained (if possible) on animals euthanized in parallel studies.
For the practising procedures described in this appendix we expect the need of 1 session per year. We foresee the need for practicing these skills for [redacted] technicians, So we expect the need of [redacted] newly ordered animals per year, which will be [redacted] animals for practicing purposes only for the coming 5 years. We expect the need of [redacted] mice and [redacted] rats in total. (See also addendum Table 1)

There is extensive experience in tumor models in mice and rats which allows the most optimal tumor growth and translation of the potency of a novel drug to human patients. The mice or rat strain used for these training purposes will be based on the animal strain needed for the particular tumor model to be established.

Details for rational of choice of different rat or mice strains are described in Appendix 2.B.

C. Re-use

Will the animals be re-used?

X No, continue with question D.

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

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

xNo

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

D. Replacement, reduction, refinement

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

The animal studies described in this appendix are used to optimize and refine the animal experiments described in appendix II (orthotopic tumor and metastasis models). By applying these practising procedures before the real establishment of the tumor model, we will enhance the success rate of the new orthotopic tumor and metastasis models.

By performing these practising studies we make sure that our dedicated personnel remains skilled. Therefore, training on a regular basis, either by participating in courses or by learning new techniques in house is essential. Also for these new techniques, specific training or dedicated external courses will increase the skills on these techniques

Furthermore the 3R's described in appendix I and II also apply for these practising studies.

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

The following measures will be taken to minimize the tumor model related discomfort:

1) The training techniques will be performed under anaesthesia, and if needed painkillers will be applied.
2) Intensive observation for clinical signs (like abnormal behaviour, posture and appearance) will be done the first days after surgery/tumor cell inoculation.
3) Only when recovery of the mice/rat is successful by means of visual clinical signs (like normal behaviour, motility, absence of piloerection), follow up monitoring can be initiated.
4) If recovery of the mice/rat is successful body weight measurements are included.
5) Tumor volumes will be monitored up to max 1 time a week by imaging when luciferase or fluorescent tumor cells are used. At the moment that tumors can be visualized by imaging, it can be validated if the tumor growth occurs in the expected organ(s). When this is confirmed, the animal will be euthanized.

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.

- For all surgeries as well as intracardiac tumor cell injections the animals will receive anesthesia
- To prevent pain, an analgesic is applied 30 minutes-1 hour before the surgery and can be repeated 24 hours after the implantation.
- The painkillers needed for more severe pain, will only be applied after consultation of the veterinarian

I. Other aspects compromising the welfare of the animals

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

NA

Explain why these effects may emerge.

-

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

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.

Since this appendix includes only practising of new techniques, it might be that mice or rats encounter discomfort/stress/complications after surgery due to the moderate skills on these certain techniques by the technician(s). However, before starting practising on the animals in this protocol, ex vivo training will be performed first by means of theoretical training and training on sacrificed animals in parallel projects.

Since tumor cells/fragments are implanted in the mice/rats, tumor growth related adverse effects can occur. However, we intend to stop the animals as soon as we see the tumors appearance and therefore it is not likely that the animals will reach the human endpoint related to tumorgrowth. But since in practising these new models we cannot fully predict the timing of tumor take appearance and since small tumors might also cause adverse effects we use the following humane endpoints as guidance:

1. Since the tumors will not be visible, imaging can be applied, in which case bioluminescent or fluorescent tumor cells are used. Based on the imaging values, we can observe the locations/organs of tumor growth and validate the expected metastasis sites of the tumor model. In additional we learn how to anticipate on the HEP in these models. HEP in these models will mostly be defined by body weight loss and clinical signs as described in point 2 and 3.
2. Body weight loss (> 20%)
3. Clinical signs, most important for orthotopical models since mostly no primary tumor is visible, are: abnormal behaviour, dyspnea (diffuclties with breathing), salivation, no response on incentives, apathy, involuntary shaking of the body or limbs (tremor), convulsion (where the body muscles contract and relax rapidly and repeatedly), self-mutilation, piloerection, abnormal non-physiological posture, paralysis, severe Diarrhea. The level of severeness of these clinical signs can be used to determine if humane HEP is reached.

When a HEP is reached animals will be directly euthanized.

Indicate the likely incidence.

Since the animal experiments in this appendix describe the practising of techniques, it might be that in several cases the animals will not recover properly from the surgery.
Incidence of reaching the HEP in relation of tumor growth can occur in some animals. However we expect

that in most cases large tumor volumes can be avoided, since the location of growth is more important in the validation of the practising then the tumor volume. Therefore we intend to stop the mice/rat before the HEP is reach caused by large tumor growth. However in some cases small tumor growth can also cause discomfort and by following the visual clinical signs we try to anticipate on and minimize the discomfort as much as possible.

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').

The procedures in general cause a moderate level of discomfort. In some cases where we euthanize the animals under anaesthesia we expect only mild discomfort. However in most cases we will monitor the recovery of the animals from anaesthesia and follow the mice/rats to check the tumor formation after 1 to 3 weeks. In some cases where the animals do not successfully recover from surgery severe discomfort can be encountered. Therefore in general we expect in the practising procedures:
In 10% mild discomfort
In 84% moderate discomfort
In 6% severe discomfort

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.

Animals are specifically bred for this procedure and they cannot be used for another type of 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

A. Algemene gegevens over de procedure

1. Aanvraagnummer : 2017.II.400.006
2. Titel van het project : Research and Evaluation of therapeutic test antibodies in tumor models in mice and rats
3. Titel van de NTS : Onderzoek en Evaluatie van therapeutische test antilichamen in kanker modellen in muizen en ratten

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

5. Contactgegevens DEC

Naam DEC	: DEC Utrecht
Telefoonnummer contactpersoon	: 088 – 75 59 247
Emailadres contactpersoon	: dec-utrecht@umcutrecht.nl

6. Adviestraject (data dd-mm-jjjj):
   - ☒ ontvangen door DEC: 03-02-2017
   - ☐ aanvraag compleet:
   - ☒ in vergadering besproken: 15-02-2017 en 15-03-2017
   - ☐ anderszins behandeld:
   - ☒ termijnonderbreking(en) van / tot : 22-02-2017/02-03-2015 en 20-03-2017/21-03-2017
   - ☐ besluit van CCD tot verlenging van de totale adviestermijn met max. 15 werkdagen:
   - ☐ aanpassing aanvraag:
   - ☒ advies aan CCD: 29-03-2017

7. De aanvraag is afgestemd met de IvD en deze is hiermee akkoord.

8. Eventueel horen van aanvrager
   - Datum: 15-03-2017
   - Plaats: Utrecht
   - Aantal aanwezige DEC-leden: 7
   - Aanwezige (namens) aanvrager: verantwoordelijk onderzoeker
   - Gestelde vragen en verstrekte antwoorden:
     Met de onderzoeker is gesproken over de selectie criteria van de targets en het doen van een terugrapportage, zie A9.

9. Correspondentie met de aanvrager
   - Datum vragen: 22-02-2017
   - Datum antwoord: 02-03-2017

- Gestelde vragen en antwoorden:
  Projectvoorstel
  - Algemeen: Tumormodellen staan ter discussie omdat men zegt dat de transleerbaarheid laag is. Kunt u aangeven wat u daarover denkt (of weet) en waarom, zodat de DEC zich een oordeel kan geven over de transleerbaarheid.
    The translatability of tumor models to cancer patients is indeed always a debate. The challenges and limitations of tumor models are often discussed at tumor model conferences and workshops and is nicely summarized by Denayer T et al., New Horizons in Translational Medicine, 2014
    However it can be mentioned that the tumor model field (including our work) is working hard on the improvements. For example Patient Derived Xenograft (PDX) models, where parts of human tumor tissues are transplanted and passaged subcutaneously in the mice result in growth of the patient derived tumor material in mice. This allows a direct analysis of the potency of anti-tumor drugs on the tumors of these patients. After several passages the structural architecture and genetics of these tumors are still very comparable to the tumor of the patients, and screening of these PDX xenograft models can be used to predict the drug response in clinical trials as is nicely described by Goa et al., Nature Medicine, 2015.
    Furthermore an enhanced translatability of tumor models can be established creating a tumor microenvironment which is more closely to the cancer patient. For example by introducing the human immune system (by means of injection of peripheral Blood Mononuclear cells) or the use of humanized mice (mice that are at a young age injected with CD34+ hematopoietic stem cells which allows differentiation of a diversity of human immune cells in these mice). Another alternative is the injection of the tumor cells in the organ of origin (orthotopic implantation). For example injection of the pancreas tumor cell line into the pancreas. This creates a "natural environment" that will be more comparable to the cancer of the patient. In addition the translational value of tumor models can be further enhanced when combined with other translational tools such as pharmacokinetic studies and biomarker studies, which is currently applied more often than in the past.
    All these improved translatable tumor models are included in our antibody development process and in this project application. But it has to be realized that the animal models will always have their limitations and cannot cover all responses of all the individual cancer patients in the clinic. Therefore, in the late phase of the development process, a strong data package (both in vitro and in vivo) is needed to convince clinicians (and patients) of the efficacy of the new anti-tumor drug, and that they are interested to enroll into the clinical trial studies. Since each tumor models differently reflects the complexity of the cancer patient, mostly more different tumor models have to be included in these data packages.
    In the early phase of the antibody drug development process, the tumor models are rather used as a bridge between the in vitro findings and the cancer of the patients, since animal tumor models better reflect the complexity of the human cancer compared to in vitro studies only. For this purpose, the tumor models described in this application are often used

in the selection process of moving the best antibody drug candidate forward (or stop the development process). We know that these tumor models have their limitations but these models are in general much more suitable for screening and selection than other models, since for some models we see less variation in tumor growth.
Overall it is important to understand the possibilities and limitations of the tumor models, and each animal tumor model that has to be performed has to be carefully selected, designed and conducted. Only then the tumor models can be used properly for the antibody drug selection process and will have translational value for clinical studies with cancer patients.

- 3.1 Achtergrond/3.4 Onderzoeksstrategie: De DEC adviseert u het projectvoorstel zo te schrijven dat alle keuzes die gemaakt worden navolgbaar zijn. De onderbouwing van hoe u komt tot de keuze voor een bepaald type tumor, of daarbij het orthotoop of subcutane model wordt gebruikt en welk type muizen daarvoor wordt ingezet mist. Hierdoor is niet te beoordelen wat de succeskans is dat voor een antibodytherapie een goed experiment is uitgevoerd. U zou bijvoorbeeld kunnen zeggen dat, waar mogelijk, voor een orthotoop model gekozen wordt om zoveel mogelijk na te bootsen wat in vivo gebeurd en als dat niet mogelijk blijkt het subcutane model wordt gebruikt. Het opnemen van een beslisboom en/of tabel waarin duidelijk staat aangegeven welk type tumor u bestudeert, welke behandeling is toegepast, welk immunologisch model u verwacht en welke muizen gebruikt worden zou de aanvraag beter navolgbaar maken en kan de kans op slagen duidelijk maken. In dit kader vraagt de DEC af of niet al lang bekend is bij welk type kanker welk model met welk type dieren geschikt is.
  As requested we added a scheme in paragraph 3.4.3, which depicts the strategy for model choice depending on the stage of the antibody development process (fig 2a). As indicated, in the early phase of the drug development process the [redacted] *In all models selected a further deviation will be made depending on the influence/need of the (human) immune system as indicated in fig 2b.*
- 3.1 Achtergrond: Het toevoegen van een afbeelding waaruit blijkt wat de antibody formats zo specifiek/bijzonder maakt zou eveneens verhelderend werken.
  A figure explaining the different antibody formats is added in 3.1, including some additional text (indicated in bleu).
- 3.1 Achtergrond: U geeft aan in principe al het onderzoek in de muis uit te voeren, behalve als er veel bloed nodig is, dan wordt de rat gebruikt. Kunnen de resultaten van de muis/rat 1-op-1 worden vertaald? Hoe gaat u daarmee om? Is het geen optie om meer muizen te gebruiken wanneer meer bloed nodig is? Graag verhelderen.
  The use of more mice is indeed an option, and this will also sometimes be the case. However for biomarker research it is an advantage to have everything from one animal, to study the cause-effect relation of the tumor growth, treatment, pharmacokinetics and biomarker research. Furthermore, by the use of more animals instead of one, more variation

is introduced which is often not preferred. In addition, the use of rats for this type of research will lead finally in to a reduction of total animal numbers.
However, the DEC is correct that it has to be considered if the mouse results can be 1:1 translated to the rat models. Therefore, by the establishments of the rat tumor models we will include one (or more) positive control antibodies, of which we know how they perform in mouse tumor models, and if this matches with the rat model. On the other hand the biomarker research will mainly be done to support the clinical trial design in patients. So, the results found in the animal study will preferable be compared to (ex vivo) research on patient materials.

- 3.1 Achtergrond: In de zin: "*Parallel to the studies described in this project application, we are studying related processes which are part of a successful antibody generation, like immunizations in rodents and pharmacokinetic studies*" graag duidelijker aangeven dat de immunisatie procedure (= de productie van antistoffen) en het onderzoek van de pharmakokinetiek onderdeel zijn/worden van aparte aanvragen/projectenbeschikkingen.
  To make this clearer I added in 3.1 the sentence: These related processes are described in two different parallel project applications: "Investigation of pharmacokinetics of antibody formats in rodents" and "Immunization of rodents (transgene for human IgG)".
- 3.1 Achtergrond: De DEC raadt u aan in de aanvraag duidelijk aan te geven dat de targets nog niet allemaal in beeld zijn.
  In 3.1 we added the text:
  "Yearly we evaluate a large number of novel potential cancer targets……
- 3.3 Belang: De zin: "*Evaluation and research of new antibodies drug candidates or new antibody formats in mice or rat tumor models will give insight in the mode of action of the drugs and the understanding of these models in cancer*". Dit roept vragen op. Wat bedoelt u met 'model' en 'understanding of these models'? Is dat het immunologisch model of het kankermodel?
  With 'understanding of these models', we meant the understanding of the applicability of all the different models for antibody efficacy studies described in this application. This indeed includes the recently developed humanized mice models, but also the orthotopic, metastasis, syngeneic, xenograft and rat models. Each model type will have its own characteristics and by studying this (for examples by taking out the tumor and explore the target expression or the immunological cell content) we will gain insight into these models and it will help to refine the future tumor models design. We adjusted the text in 3.3 slightly to make it more clear: "Furthermore, by analyzing the different animal tumor models described in this application, we will gain understanding in the use…….

Bijlage 1

- Experimentele aanpak en primaire uitkomstparameters/bijlage I: De DEC is heeft moeite met het begrijpen van de powerberekening in addendum I: Power analyses. In de figuur worden groeicurves getoond met een [redacted]. In de

tabel staan de gemiddelde volumina van dezelfde tumoren, maar nu met de een standaard deviatie. Voor de powerberekening gaat u uit van het eindvolume op de laatste dag dat die groep een intacte tumor heeft. Daarbij gaat u voorbij aan het feit dat dat eind volume op verschillende dagen valt. Zou u kunnen toelichten waarom u de [redacted] Graag uw toelichting/visie.

In the graphs of addendum I, we display the curves of only the control group from 5 different independent experiments which all were performed using the subcutaneous inoculation of A431 in immune deficient (SCID) mice or B16F10 tumor cells in immune competent C57/bl6 mice. [redacted] *By performing both these statistical analysis we believe we support the observed tumor growth data in the most representative way in most studies to be performed within this project application.*

The methods the DEC raises (linear regression on logarithmic scale and the area under the curve (AUC)) are indeed alternative methods which are more and more discussed. [redacted]

[redacted]

We do, however, keep exploring the most optimal analysis methods including the 'area under the curve' (AUC) and the linear regression. But at this moment these analysis are still exploratory in the field, and it cannot be stated (yet) that another method is better than the ones that we are currently using.

- Experimentele aanpak en primaire uitkomstparameters: De DEC geeft u de suggestie om het opzetten van mogelijk nieuwe tumormodellen te beschrijven in een nieuwe bijlage.
  We considered the splitting of the "to be established tumor models" in a separate appendix, However, since it concerns both the establishment of new models in the appendix I (s.c. tumor models) and in Appendix 2 (orthotopic and metastasis modes), we were not convinced that a new appendix would provide more clarity in the application. Furthermore, the handlings described in the two appendices both also are incorporated in the establishment of the new models, the only thing which is different in the establishment, is the lack of antibody treatment.
  To give more insight in the "models to be established", we have chosen to adjust both applications 1 and 2 by adding a more detailed description of new models to be established. See changes in green throughout appendix 1 and 2). Furthermore to provide more insight in the expected numbers of these experiments we will add Table 1 with the expected number of animals. This gives an overview of the total number we expect to use for the different appendixes including the efficacy models and establishment models. These numbers are also included in 2B. We think this gives a better insight in the expected establishment of new tumor models.
- Experimentele aanpak en primaire uitkomstparameters: U geeft aan dat het af en toe nodig is een medewerker te trainen in de modellen. De DEC adviseert u ook hiervoor een aparte bijlage schrijven omdat dat meer transparantie geeft.
  As requested we made a separate appendix for this and is now described in a new appendix 3 "Practicing of several techniques for orthotopic tumor and metastasis tumor models"
- B. De dieren: Het is niet helder waar het aantal geschatte experimenten en het aantal van [redacted] dieren per experiment op gebaseerd is. De aantallen die nodig zijn voor het opzetten

van nieuwe modellen en voor training is ook niet terug te vinden, maar dit wordt ondervangen door de extra bijlage voor deze nieuwe modellen.
The number of animals needed is for training is also included in Table 1 as well as the rational for the number of animals needed per experiments, needed for in the s.c. tumor models and the orthotopic and metastasis models. Including the animals expected for the establishment of new tumor models.

- J. Humane eindpunten: De DEC verzoekt u hier nog toe te voegen dat u in de meeste gevallen 1500 mm3 hanteert en niet de 2000 mm3 uit de Code of Practice. Dit geldt ook voor bijlage 2.
  We do want to stress that we do not consider a tumor volume of 1500 mm3 as a human endpoint. We do indeed have our internal policy in stopping the mice when a tumor volume equal or higher than 1500mm3 is detected. This we apply because the animals in our studies are euthanized always at the end of the study, and since in most of our studies no additional scientific values will be obtained at tumor sizes above 1500mm3. Therefore, we intend to stop the mice BEFORE the human endpoint of 2000 mm3 is reached, and use > 1500mm3 as an internal guideline. Subcutaneous tumors are inoculated at the flank of the mice and mostly do not seem to have severe discomfort of a tumor size up to 2000 mm3. In our 15 years' experience we observed that the animals are viable and have no additional clinical signs and are still very mobile and active even if a tumor size of 2000mm3 is reached. We therefore follow the 'Code of Practice' guidelines and use the 2000 mm3 for the mice as a human endpoint and not the 1500mm3. We apply a scoring of discomfort of the mice at a tumor size up to 1000 mm3 as mild, a tumor size between 1000-2000mm3 as moderate and above 2000mm3 as severe discomfort. We did add the text, that we in most cases stop the mice when a tumor volume of higher than 1500 mm3 is detected.

Bijlage 2

- K. Classificatie van ongerief: Hier worden exact dezelfde percentages ongerief aangegeven als in bijlage 1. De DEC vraagt zich af of dit klopt. Graag verhelderen.
  We did indeed use the same number since it is an estimation of what we expect. But the DEC is correct that we might have to adjust this a bit. The animal models described in appendix I are the most experiments to be performed in the coming years, where we have the most experience with and which we can rate the best. So, there we expect a discomfort of 22, 75 and 3%. The handlings in the orthotopic and metastasis models are more complex than the subcutaneous tumor models and need in most cases repeated imaging and in some cases surgery. These handlings are all done under anesthesia, which will reduce the discomfort of the mice/rats but we re-discussed and came to the conclusion that repeated anesthesia and recovery of surgery would also give moderate discomfort. So we expect more moderate discomfort in this appendix and adjusted this to 10, 87 and 3 %. In the appendix describing the practicing of new techniques we expect indeed some more severe discomfort because of learning the technical skills of these techniques. Although we perform these

studies also under anesthesia, use painkillers where needed, and stop the animal at an earlier stage of the study, we have adjusted the expected level to 10, 84 and 6%.

- De antwoorden hebben geleid tot aanpassing van de aanvraag.

- Datum vragen: 20-03-2017
- Datum antwoord: 21-03-2017
- Gestelde vragen en antwoorden:
  - De DEC raadt u aan een omschrijving van de selectiecriteria van de targets op te nemen in het projectvoorstel en te noemen dat u desgewenst bereid bent een jaarlijkse terugrapportage te verstrekken.
    In the previous version we did already mention more about the target selection process. But since this is again specifically mentioned by the DEC, we elaborate more on this in section 3.1 of the study proposal. Furthermore as discussed, we will indicate to the CCD that if desired, we are open for reporting back the progress of the application.
- De antwoorden hebben geleid tot aanpassing van de aanvraag.

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

B. Beoordeling (adviesvraag en behandeling)

1. Het project is vergunningplichtig (dierproeven in de zin der wet).
2. De aanvraag betreft een nieuwe aanvraag.
3. De DEC is competent om hierover te adviseren.
4. Er zijn geen DEC-leden betrokken bij het betreffende project.

C. Beoordeling (inhoud):

1. De aanvraag is toetsbaar en heeft voldoende samenhang.
   Een vrij recente en succesvolle therapievorm voor patiënten met kanker is de toepassing van therapeutische antilichamen. Om deze therapievorm te optimaliseren en breder toepasbaar te maken worden nieuwe en gemodificeerde therapeutische antilichamen ontwikkeld. De aanvrager wil met behulp van de voorliggende projectaanvraag het werkingsmechanisme en de effectiviteit van nieuwe en gemodificeerde therapeutische antilichamen in muizen en ratten in kaart brengen, zodat met behulp van de verkregen resultaten de meest veelbelovende antilichamen geselecteerd kunnen worden voor verder (pre)klinisch onderzoek. Het belang van deze doelstelling is in de ogen van de DEC substantieel.

De relatie tussen het hoofddoel en de subdoelen komt – voor wat betreft de bijlagen 1 en 2 – overeen met voorbeeld 4B uit de 'Handreiking Invulling Definitie Project'. Bijlage 3 is een essentieel onderdeel van de projectaanvraag, dat nodig is om de experimenten van bijlage 2 te kunnen doen slagen.

2. Voor zover de DEC bekend, is er geen mogelijk tegenstrijdige wetgeving die het uitvoeren van de dierexperimenten in de weg zou kunnen staan.

3. De in de aanvraag aangekruiste doelcategorie sluit aan bij de hoofddoelstellingen.

Belangen en waarden

4. Het directe doel van het project is de selectie, analyse en evaluatie van nieuwe therapeutische antilichamen met behulp van *in vivo* tumormodellen in muizen en ratten. Het uiteindelijke doel van het project is de ontwikkeling van nieuwe therapeutische antilichamen voor de behandeling van kankerpatiënten. Voordat de veiligheid en effectiviteit van dergelijke antilichamen in klinische trials in mensen onderzocht kunnen worden is het van belang dat allerlei belangrijke aspecten (zoals het werkingsmechanisme en de effectiviteit) nauwkeurig in kaart gebracht worden met behulp van *in vitro* en dierexperimenten. De DEC is daarom van mening dat er in voldoende mate een relatie is tussen het directe doel en het uiteindelijke doel.

5. De belangrijkste belanghebbenden in dit onderzoeksproject zijn de proefdieren, de doelgroep (kankerpatiënten), het onderzoeksveld en de vergunninghouder. De morele waarden die voor de proefdieren in het geding zijn: welzijn (gezondheid en stress) en rechtvaardigheid (intrinsieke waarde en integriteit). De morele waarden die voor de doelgroep worden bevorderd zijn: welzijn (kwaliteit van leven) en rechtvaardigheid (beschikbaarheid van een doeltreffende therapeutische behandeling). De morele waarden die voor het onderzoeksveld en de vergunninghouder worden bevorderd zijn: welzijn (wetenschappelijke en commerciële ontwikkelingen).

6. De aanvrager geeft niet aan nadelige effecten op het milieu te verwachten. De DEC ziet geen aanleiding om aan te nemen dat zich toch nadelige effecten zullen voordoen.

Proefopzet en haalbaarheid

7. De kennis en kunde van de onderzoeksgroep en andere betrokkenen bij de dierproeven zijn voldoende gewaarborgd en dragen eraan bij dat de doelstellingen behaald kunnen worden, dat aan de 3V-beginselen voldaan kan worden en dat voorkomen kan worden dat mens, dier en milieu negatieve effecten ondervinden als gevolg van de dierproeven. De onderzoeksgroep heeft veel ervaring met de ontwikkeling van therapeutische antilichamen en de uitvoering van het dierexperimenteel onderzoek dat daar een belangrijk onderdeel van is. De onderzoeksgroep heeft in het verleden laten zien dat preklinische studies, zoals beschreven in de voorliggende projectaanvraag, eraan bijdragen dat nieuwe therapeutische antilichamen succesvol naar de

kliniek gebracht kunnen worden. De DEC is van mening dat het projectvoorstel aansluit bij recente inzichten en dat het geen belangrijke hiaten bevat die de bruikbaarheid van de resultaten beperken.

8. Het project is goed opgezet, de voorgestelde experimentele opzet en uitkomstparameters sluiten logisch en helder aan bij de aangegeven doelstellingen en de gekozen strategie en experimentele aanpak kan leiden tot het behalen van de doelstelling binnen het kader van het project. In dit project wordt de effectiviteit van verschillende therapeutische antilichamen onderzocht in muizen en ratten (bijlage 1 en 2), waarbij de opzet van de experimenten kan variëren en is afgestemd op het werkingsmechanisme van het betreffende antilichaam, de target het type en de lokalisatie van de tumor waartegen het antilichaam wordt ingezet) en de bijbehorende onderzoeksvragen. Er wordt gebruik gemaakt van subcutane tumormodellen (bijlage 1) en orthotope en metastatische tumormodellen (bijlage 2). Met de meeste tumormodellen heeft de onderzoeksgroep reeds ruimschoots ervaring, maar het is ook mogelijk dat voor een bepaald antilichaam en bijbehorende target een nieuw tumormodel opgezet moet worden. Om de bekwaamheid van alle betrokkenen te waarborgen worden medewerkers getraind in het uitvoeren van twee experimentele handelingen, die essentieel zijn voor het slagen van de experimenten met de orthotope en metastatische tumormodellen in muizen en ratten (bijlage 3). De keuze voor de te testen antilichamen is gebaseerd op resultaten van *in vitro* assays waarin de specifieke bindings- en functionele activiteit van de antilichamen onderzocht is. De wijze waarop de keuze voor een bepaald tumormodel tot stand komt is met behulp van figuur 2 in de projectbeschrijving helder weergegeven.

Welzijn dieren

9. Er is 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 EU richtlijn)
   ☐ Zwerfdieren (10h)
   ☒ Hergebruik (1e lid 2)
   ☐ Locatie: buiten instelling vergunninghouder (10g)
   ☐ Geen toepassing verdoving/pijnbestrijding (13)
   ☐ Dodingsmethode niet volgens bijlage IV EU richtlijn (13c lid 3)
   De keuze hiervoor is voldoende wetenschappelijk onderbouwd en de aanvrager voldoet aan de in de Wet op de dierproeven, voor de desbetreffende categorie, genoemde beperkende voorwaarden. Muizen en ratten kunnen hergebruikt worden voor trainingsdoeleinden (bijlage 3) of voor de zogenaamde *re-challenge* en *resistance* studies (bijlagen 1 en 2).

10. De dieren worden gehuisvest en verzorgd op een wijze die voldoet aan de eisen die zijn opgenomen in bijlage III van de EU richtlijn.

11. Het cumulatieve ongerief als gevolg van de dierproeven is realistisch ingeschat en geclassificeerd. Voor bijlage 1 schat men in dat 22% van dieren licht ongerief ervaart, 75% matig ongerief en 3% ernstig ongerief. Dit ongerief is het gevolg van verschillende experimenten handelingen en de bijverschijnselen van de geïnduceerde tumorgroei. Wanneer geen tumorgroei plaatsvindt blijft het ongerief beperkt tot licht ongerief. Voor bijlage 2 schat men in dat 10% van de dieren licht ongerief ervaart, 87% matig ongerief en 3% ernstig ongerief. In vergelijking met bijlage 1 zullen meer dieren matig ongerief ervaren, omdat de aard van de modellen (orthotope en metastasemodellen) meer/complexere experimentele handelingen vereisen. Voorbeelden van dergelijke handelingen zijn chirurgische benadering van organen ten behoeve van tumorinductie en herhaaldelijke anesthesie ten behoeve van beeldvorming. Voor bijlage 3 schat men in dat 10% van dieren licht ongerief ervaart, 84% matig ongerief en 6% ernstig. Laatstgenoemde categorie ongerief is hoger dan in de andere bijlagen, omdat experimentele handeling door minder ervaren medewerkers worden uitgevoerd in het kader van training. Alle inschattingen zijn gebaseerd op jarenlange ervaring met vergelijkbare experimenten.

12. De integriteit van de dieren wordt fysiek en mentaal aangetast. Fysieke aantasting is het gevolg van geïnduceerde tumorgroei en verschillende experimentele handelingen, zoals chirurgie en bloedafnamen. Mentale aantasting is het gevolg van (herhaaldelijke) anesthesie die vereist is voor een aantal experimentele handelingen, zoals chirurgie en beeldvorming.

13. De humane eindpunten zijn in de bijlage dierproeven goed gedefinieerd en het percentage dieren dat naar verwachting een humaan eindpunt bereikt is goed ingeschat. De aanvrager heeft, zowel schriftelijk als mondeling, duidelijk toegelicht wat in de experimenten met muizen het verschil is tussen het experimenteel eindpunt (een tumorvolume van 1500 mm3) en het humaan eindpunt (een tumorvolume van 2000 mm3). Wanneer een tumor een volume van 1500 mm3 heeft bereikt, dan wordt het betreffende dier geëuthanaseerd, omdat het geen aanvullende wetenschappelijk relevante informatie zou opleveren wanneer men het dier in leven zou laten (experimenteel eindpunt). Men ziet erop toe dat het tumorvolume de grens van 2000 mm3, die conform de 'Code of Practice' als humaan eindpunt gehanteerd wordt, niet overschrijdt, zodat het ongerief ten gevolge van de geïnduceerde tumoren beperkt blijft tot matig ongerief. Voor ratten hanteert men conform de 'Code of Practice' een tumorvolume van 40cm3 en een diameter van 4,2 cm als humaan eindpunt. Naast de afmetingen van de tumoren worden ook een groot aantal andere verschijnselen als humaan eindpunt gehanteerd. Daarbij gaat het om een breed scala aan verschijnselen, zoals gewichtsverlies, ulceratie of paralyse, die het gevolg kunnen zijn van tumorgroei. Men houdt er rekening mee dat in de bijlage 1, 2 en 3 respectievelijk 3%, 3% en 6% van de dieren het humaan eindpunt bereikt. Dit komt overeen met de percentages dieren die naar verwachting ernstig ongerief zullen ervaren (zie C11).

3V's

14. De aanvrager heeft voldoende aannemelijk gemaakt dat er geen geschikte vervangingsalternatieven zijn. De effectiviteit van therapeutische antilichamen is in grote mate afhankelijk van – deels nog onbekende – complexe interacties tussen verschillende organen, weefsels en cellen. Daarbij gaat het bijvoorbeeld om het zogenaamde *microenvironment* van de tumor en de activiteit van het immuunsysteem. Dergelijke factoren kunnen niet in hun volledigheid *in vitro* of *in silico* nagebootst worden. *In vitro* experimenten en *in silico* simulaties worden wel gebruikt om een voorselectie te maken van veelbelovende therapeutische antilichamen, en om verkregen resultaten te vergelijken/valideren met eerder uitgevoerde experimenten en gegevens uit de literatuur.

15. Het aantal te gebruiken dieren is realistisch ingeschat en er is een heldere strategie om ervoor te zorgen dat tijdens het project met zo min mogelijk dieren wordt gewerkt waarmee een betrouwbaar resultaat kan worden verkregen. De aanvrager heeft – in de bijlagen beschrijving dierproeven en met behulp van meerdere addenda – zeer uitvoerig uiteengezet op welke wijze de juiste groepsgroottes voor de verschillende experimenten en experimentele groepen worden bepaald. De groepsgrootte zal onder andere afhangen van het type experiment (exploratief vs. kwantitatief), het type tumor en tumorgroei (homogeen vs. heterogeen) en de uitleesparameters.

16. Het project is in overeenstemming met de vereiste van verfijning van dierproeven en het project is zodanig opgezet dat de dierproeven zo humaan mogelijk kunnen worden uitgevoerd. De onderzoeksgroep heeft veel ervaring met de uit te voeren experimentele handelingen en metingen, en kan de snelheid en aard van de tumorgroei en de te verwachten bijverschijnselen in de verschillende modellen goed inschatten (uiteraard met uitzondering van eventueel nieuw op te zetten modellen). De dieren worden nauwlettend geobserveerd, zodat tijdig geconstateerd wordt wanneer een dier het humaan eindpunt bereikt, en het optreden van ernstig ongerief tot een minimum beperkt kan worden.

17. Er is geen sprake van wettelijk vereist onderzoek.

Dieren in voorraad gedood en bestemming dieren na afloop proef

18. Dieren van beide geslachten zullen niet in gelijke mate worden ingezet. Voor de bijlagen 1 en 2 zullen met name vrouwelijke dieren ingezet worden, omdat de activiteit van het complementsysteem in mannelijke dieren anders is dan in vrouwelijke dieren. Daardoor kan de effectiviteit van de antilichamen tussen de beide geslachten verschillen. Door dieren van één geslacht in te zetten kan men de variatie binnen en tussen experimenten – en daarmee het aantal dieren per experimentele groep – minimaliseren. De keuze voor vrouwelijk dieren heeft als bijkomende voordeel dat de dieren makkelijker in groepen gehuisvest kunnen worden. Daar waar de aard van een experiment het toelaat (doordat metingen gericht zijn op het individuele dier), zullen wél zowel mannelijke als vrouwelijke dieren ingezet worden. Er worden ook

experimenten uitgevoerd waarbij de locatie van de tumor vraagt om inzet van enkel mannelijke dieren (experimenten met tumoren in de prostaat). In bijlage 3 zullen beide geslachten ingezet worden voor de trainingsdoeleinden. De DEC is ervan overtuigd dat de aanvrager in voldoende mate wetenschappelijk heeft onderbouwd dat het, om de doelstelling te bereiken, in een groot deel van de experimenten noodzakelijk is om de proeven met
of alleen mannelijke of alleen vrouwelijke dieren uit te voeren.

19. De dieren worden in het kader van het project gedood, omdat de doelstelling van het project alleen behaald kan worden wanneer tumoren en anderen weefsels post mortem geanalyseerd worden. Bovendien zou het niet toelaatbaar zijn om dieren met geïnduceerde tumorgroei na afloop van het experiment in leven te laten. De dieren worden volgens een passende – en in bijlage IV van de EU richtlijn genoemde – methode gedood.

20. Omdat in het projectvoorstel muizen en ratten worden aangevraagd is de vraag over herplaatsing/hergebruik niet van toepassing.

NTS

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

D. Ethische afweging

1. De morele vraag die de DEC dient te beantwoorden is: rechtvaardigt het belang van het voorliggende project, dat bijdraagt aan de ontwikkeling van nieuwe therapeutische antilichamen voor de behandeling van kankerpatiënten, de onvermijdelijke aantasting van het welzijn en de integriteit van de gebruikte proefdieren?

2. Er vindt een aanzienlijke aantasting van welzijn en integriteit van de proefdieren plaats, welke gepaard gaat met licht tot ernstig ongerief. Indien de hierboven genoemde doelstellingen behaald worden, dan zal dit project eraan bijdragen dat nieuwe en effectievere therapeutische antilichamen beschikbaar komen voor kankerpatiënten. Patiënten zouden daar zeer bij gebaat zijn, omdat de huidige therapieën vaak onvoldoende effectief zijn en gepaard gaan met vervelende bijverschijnselen. Het is aannemelijk dat de translationele doelstelling behaald zal worden. Daarvoor is de inzet van proefdieren noodzakelijk, maar de onderzoekers doen al het mogelijke om het ongerief voor de dieren en het aantal dieren tot een minimum te beperken.

3. Op grond van het bovenstaande is de DEC van oordeel dat de selectie, analyse en evaluatie van nieuwe therapeutische antilichamen met behulp van *in vivo* tumormodellen in muizen en ratten een substantieel belang vertegenwoordigt en dat dit substantiële belang opweegt tegen de aanzienlijke aantasting van het welzijn en de integriteit van de proefdieren. Het gebruik van de proefdieren zoals beschreven in de aanvraag is daarmee gerechtvaardigd.

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.

   ☐ De DEC adviseert de vergunning niet te verlenen vanwege:

2. Het uitgebrachte advies is gebaseerd op consensus.

3. Er zijn geen knelpunten/dilemma's naar voren gekomen tijdens het beoordelen van de aanvraag en het opstellen van het advies.

8.

Centrale Commissie Dierproeven

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0900 28 000 28 (10 ct/min)
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Onze referentie
Aanvraagnummer
AVD2440020171264

Bijlagen
2

Datum 3 april 2017
Betreft Ontvangstbevestiging aanvraag projectvergunning Dierproeven

Geachte mevrouw [redacted]

Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 31 maart 2017. Het gaat om uw project "Research and Evalution of therapeutic test antibodies in tumor models in mice and rats". Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD2440020171264. 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:
3 april 2017
Aanvraagnummer:
AVD2440020171264

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlagen:
- Gegevens aanvraagformulier
- Factuur

Datum:
3 april 2017
Aanvraagnummer:
AVD2440020171264

Gegevens aanvrager

Uw gegevens

Deelnemersnummer NVWA:	24400
Naam instelling of organisatie:	Genmab B.V.
Naam portefeuillehouder of diens gemachtigde:	[redacted]
KvK-nummer:	30169902
Straat en huisnummer:	Yalelaan 60
Postcode en plaats:	3584 CM UTRECHT
IBAN:	[redacted]
Tenaamstelling van het rekeningnummer:	[redacted]

Gegevens plaatsvervangende verantwoordelijke onderzoeker

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

Datum:
3 april 2017
Aanvraagnummer:
AVD2440020171264

Gegevens gemachtigde	
KvK-nummer:	30169902
Naam:	[redacted]
Adres:	Yalelaan 60
Postcode en plaats:	3508 AD UTRECHT
Wilt u een nieuwe machtiging afgeven?	Ja
Wat mag de gemachtigde doen?	[ ] Een projectvergunning aanvragen [ ] Een wijziging op een verleende projectvergunning aanvragen [ ] Een melding doorgeven op een verleende projectvergunning [ ] Een bezwaarschrift indienen en daarover communiceren met de Centrale Commissie Dierproeven en alle andere handelingen verrichten die nodig zijn voor een goede afwikkeling van het bezwaarschrift [x] Alle bovenstaande opties

Over uw aanvraag

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

Over uw project

Geplande startdatum:	1 mei 2017
Geplande einddatum:	1 mei 2022
Titel project:	Research and Evalution of therapeutic test antibodies in tumor models in mice and rats
Titel niet-technische samenvatting:	Onderzoek en Evaluatie van therapeutische test antilichamen in kanker modellen in muizen en ratten
Naam DEC:	Utrecht
Postadres DEC:	Bureau van de DEC Utrecht , Huispostnummer D01.343, Postbus 85500, 3508 GA Utrecht
E-mailadres DEC:	dec-utrecht@umcutrecht.nl

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] Melding Machtiging [x] DEC-advies

Ondertekening

Naam:	[redacted]
Functie:	[redacted]
Plaats:	Utrecht
Datum:	30 maart 2017

Centrale Commissie Dierproeven

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Onze referentie
Aanvraagnummer
AVD2440020171264

Bijlagen
2

Datum 3 april 2017
Betreft Factuur aanvraag projectvergunning Dierproeven

Factuur
Factuurdatum: 3 april 2017
Vervaldatum: 3 mei 2017
Factuurnummer: 171264

Omschrijving	**Bedrag**
Betaling leges projectvergunning dierproeven Betreft aanvraag AVD2440020171264	€ 1.541,00

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

Centrale Commissie Dierproeven

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Onze referentie
Aanvraagnummer
AVD2440020171264

Datum 14 april 2017
Betreft Aanvulling aanvraag projectvergunning Dierproeven

Geachte mevrouw [redacted],

Op 31 maart 2017 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Research and Evalution of therapeutic test antibodies in tumor models in mice and rats" met aanvraagnummer AVD2440020171264. In uw aanvraag zitten voor ons nog enkele onduidelijkheden. In deze brief leest u wat wij nog nodig hebben en wanneer u een beslissing kunt verwachten.

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

Niet technische samenvatting
De NTS bevat enkele voor het brede publiek moeilijk te begrijpen termen, zoals recombinante antilichamen en imaging. Graag hier in de NTS andere termen voor gebruiken of de termen (kort) uitleggen.
Daarnaast is in 3.5 niet helder hoeveel dieren licht, matig dan wel ernstig ongerief zullen krijgen. Graag dit verhelderen.

Onduidelijkheden
1) U heeft als doelcategorie translationeel of toegepast onderzoek aangekruist. Naar ons idee beschrijft u in uw aanvraag ook fundamenteel onderzoek (MoA studies bijvoorbeeld) maar ook onderwijs/opleiding (bijlage 3.4.4.3). Graag deze doelcategorieën toevoegen aan projectvoorstel en NTS of onderbouwen waarom u niet voor deze categorieën gekozen heeft.
2) In bijlagen 3.4.4.1 en 3.4.4.2 beschrijft u hergebruik van dieren voor meerdere doeleinden. Er is sprake van hergebruik van een dier wanneer voor een vervolgproef in plaats van het betreffende dier evengoed een ander dier

gebruikt kan worden. De voorbeelden die u noemt (re-challenge experimenten en resistance studies) vallen hier niet onder omdat hier niet een willekeurig ander dier voor gebruikt zou kunnen worden. Graag dit aanpassen.
3) In de verschillende bijlagen beschrijft u een acclimatisatieperiode van tenminste 3 dagen. Algemeen geaccepteerd wordt een acclimatisatieperiode van tenminste 1 week. Graag dit aanpassen.
4) In de bijlagen 3.4.4.1 en 3.4.4.2 beschrijft u een aantal handelingen die mogelijk bij de dieren zouden kunnen worden toegepast. Het is niet helder in welke combinaties deze handelingen zouden kunnen voorkomen en hoeveel handelingen maximaal bij elk dier zouden kunnen voorkomen. Graag dit verhelderen, eventueel in tabelvorm.
5) Onder vraag L van de bijlagen beschrijft u dat de dieren gedood worden omdat "Animals are specifically bred for this procedure and they cannot be used for another type of experiment". Het is niet duidelijk wat hiermee bedoeld wordt aangezien de muizenstammen die u gebruikt in onze visie wel voor andere typen onderzoek gebruikt zouden kunnen worden. Graag dit verhelderen.
6) In bijlagen 3.4.4.1 en 3.4.4.2 beschrijft u een voorkeur te hebben voor vrouwelijke dieren. Aangezien uw belangrijkste doelcategorie van het project translationeel onderzoek is, en kanker bij de mens in beide geslachten voorkomt, lijkt het gebruik van beide geslachten wenselijk (m.u.v. de geslachtsspecifieke tumoren uiteraard). Indien onvoldoende helder is waarom geen gebruik gemaakt wordt van beide geslachten, kan de CCD hier een voorwaarde over opnemen die gebruik van beide geslachten voorschrijft. Kunt u helderder aangeven waarom u toch voor vrouwelijke dieren kiest en hoeveel extra dieren het gebruik van beide geslachten zou kosten als gevolg van de grotere spreiding?
7) Bijlage 3 4.4.3 beschrijft oefenen van technieken en in bijlagen 3.4.4.1 en 3.4.4.2 wordt beschreven dat er jaarlijks nieuwe modellen worden ontwikkeld. Het onderscheid in modelontwikkeling en oefenen van technieken lijkt in de beschrijving een beetje door elkaar te lopen. Als bijlage 3.4.4.3 alleen om training van de technieken gaat, is het niet helder waarom in bijlage 3.4.4.3 gekozen kan worden voor het in leven laten van de dieren tot 16 weken, met potentieel ernstig ongerief. Wanneer het enkel gaat om oefenen van technieken zouden de dieren ook eerder kunnen worden gedood. Zoals het nu beschreven staat lijkt dit dus ook op modelontwikkeling. Om een en ander te verhelderen zou u, indien nodig, ervoor kunnen kiezen om alle modelontwikkeling en training in bijlage 3.4.4.3 te beschrijven.

Leges
De leges die u verschuldigd bent zijn nog niet door ons ontvangen of de betaling is nog niet verwerkt. Uw aanvraag is niet compleet als de leges niet zijn ontvangen.
Zonder deze aanvullende informatie kan de beslissing nadelig voor u uitvallen omdat de gegevens onvolledig of onduidelijk zijn.

Datum:
14 april 2017
Aanvraagnummer:
AVD2440020171264

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

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

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

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlagen:
- Melding bijlagen
- Niet technische samenvatting

10.

Date: 20 april 2017
Letter and reply project application AVD2440020171264

Dear CCD,

Thank you for your review and your remarks on the application. Below you can find your questions in black and the answers in blue. The adaptations in official application forms we made in red (except for the NTS), to provide good insight into the changes in response to your remarks and questions. Since we have in the Animal Welfare Body of Genmab English speaking people, we answered your questions in English, because of our internal reviewing process.

Hopefully this provides you a good insight and helps you further in your reviewing process.

Best regards,
AWB Genmab.

Questions and Answers:

Niet technische samenvatting
De NTS bevat enkele voor het brede publiek moeilijk te begrijpen termen, zoals recombinante antilichamen en imaging. Graag hier in de NTS andere termen voor gebruiken of de termen (kort) uitleggen.
Daarnaast is in 3.5 niet helder hoeveel dieren licht, matig dan wel ernstig ongerief zullen krijgen. Graag dit verhelderen.

The NTS is adapted,
We adjusted the term "recombinant antilichamen" to: *"antilichamen, die in het laboratorium specifiek gemaakt zijn om de kankercel te herkennen"*

We adjusted section 3.5, changed the term imaging to *"tumor volume metingen onder anesthesie"*, and made the description of the discomfort of the total animals more clear as follow:
"Dieren die sterk geremde of geen tumor ontwikkeling laten zien zullen een licht ongerief ondervinden. Dit wordt verwacht in 20% van het totaal aantal dieren.
Het grootste deel van de dieren (77%) zal matig ongerief ondervinden door tumorgroei gerelateerde ontwikkelingen, door herhaaldelijke tumorvolume metingen onder anesthesie, of bij herstel uit een operatie onder anesthesie.
In sommige gevallen, bijvoorbeeld bij een snel groeiende tumor, of bij het oefenen van nieuwe technieken kan het ongerief op ernstig uit komen. We schatten in dat dit ongeveer bij 3 % van de dieren kan voor komen"

Onduidelijkheden

1) U heeft als doelcategorie translationeel of toegepast onderzoek aangekruist. Naar ons idee beschrijft u in uw aanvraag ook fundamenteel onderzoek (MoA studies bijvoorbeeld) maar ook onderwijs/opleiding (bijlage 3.4.4.3). Graag deze doelcategorieën toevoegen aan projectvoorstel en NTS of onderbouwen waarom u niet voor deze categorieën gekozen heeft.

We indicated "translational research" since this is the general goal of the whole project application. But the CCD is correct that we also perform animal studies to analyse mechanism of action of the antibodies. Also, we do use animals specifically for training purposes as indeed is described in 3.4.4.3. Therefore, we follow the advice of the CCD and mark the category "Basic research and Higher education" as well as "Translational or applied research". We have adjusted this in the project proposal and in the NTS.

2) In bijlagen 3.4.4.1 en 3.4.4.2 beschrijft u hergebruik van dieren voor meerdere doeleinden. Er is sprake van hergebruik van een dier wanneer voor een vervolgproef in plaats van het betreffende dier evengoed een ander dier gebruikt kan worden. De voorbeelden die u noemt (re-challenge experimenten en resistance studies) vallen hier niet onder omdat hier niet een willekeurig ander dier voor gebruikt zou kunnen worden. Graag dit aanpassen.

Thanks for this advice, we did not realize this.
In both 3.4.4.1 and 3.4.4.2 we adjusted in 'C' the sentence into the following:
'Most mice/rats will not be re-used.'
However, in some experiments we may consider to use some animals for training purposes (injections mainly) before the animals will be euthanized.

3) In de verschillende bijlagen beschrijft u een acclimatisatieperiode van tenminste 3 dagen. Algemeen geaccepteerd wordt een acclimatisatieperiode van tenminste 1 week. Graag dit aanpassen.

We have adjusted this in all three appendixes to 1 week.

4) In de bijlagen 3.4.4.1 en 3.4.4.2 beschrijft u een aantal handelingen die mogelijk bij de dieren zouden kunnen worden toegepast. Het is niet helder in welke combinaties deze handelingen zouden kunnen voorkomen en hoeveel handelingen maximaal bij elk dier zouden kunnen voorkomen. Graag dit verhelderen, eventueel in tabelvorm.

To make this more clear, we created a table in which we indicated the different handling steps needed in the most typical tumor models/procedures we are intending to perform within the different animal procedures.

Please see Addenda: 5e 2017.II.400.006 Table 2 overview handling different model types

5) Onder vraag L van de bijlagen beschrijft u dat de dieren gedood worden omdat "Animals are specifically bred for this procedure and they cannot be used for another type of experiment". Het is niet duidelijk wat hiermee bedoeld wordt aangezien de muizenstammen die u gebruikt in onze visie wel voor andere typen onderzoek gebruikt zouden kunnen worden. Graag dit verhelderen.

We meant that these animals are only used for this experiment and not for other experiments. This is because almost all mice or rats receive tumor cells in our experiments, and it is not justified to use the animals for follow-up experiments. All those animals are killed at the end of the experiment.

In all animal procedures we have changed the sentence in 'L' now to: *"Animals are specifically ordered for this procedure and they cannot be used for another type of experiment since they have been injected with tumor cells"*

6) In bijlagen 3.4.4.1 en 3.4.4.2 beschrijft u een voorkeur te hebben voor vrouwelijke dieren. Aangezien uw belangrijkste doelcategorie van het project translationeel onderzoek is, en kanker bij de mens in beide geslachten voorkomt, lijkt het gebruik van beide geslachten wenselijk (m.u.v. de geslachtsspecifieke tumoren uiteraard).
Indien onvoldoende helder is waarom geen gebruik gemaakt wordt van beide geslachten, kan de CCD hier een voorwaarde over opnemen die gebruik van beide geslachten voorschrijft.
Kunt u helderder aangeven waarom u toch voor vrouwelijke dieren kiest en hoeveel extra dieren het gebruik van beide geslachten zou kosten als gevolg van de grotere spreiding?

We understand the remark from the CCD to use both male and female animals, since our focus of the experiments is "translational research". It is indeed the case that there are differences in both genders of the mice, as is described in several publications. However, it is under debate that the sex difference in mice is directly translatable to human diseases or results into a better translation into clinical studies.
A very interesting article on this topic "How Much Do Sex Differences Matter in Mouse Studies?" has been published very recently by Joshua A. Krisch in The Scientist (24 Feb 2017) (http://www.the-scientist.com/?articles.view/articleNo/48616/title/How-Much-Do-Sex-Differences-Matter-in-Mouse-Studies-/).This article exactly describes the issues we are struggling with to use both genders in our studies. To perform analysis on both sexes, you need double amount of animals and, thereby, this doubles your effort, doubles your time and doubles the costs. And, this investment does not automatically provide us with good arguments that this strategy leads to a better translation into human studies.
Besides this (and already discussed in the application), females are much easier to handle and are less aggressive, whereby separate housing can be avoided, which is often the case by male animals and leads to real stress for the animals. In addition, we have a rather long experience of tumor models using only female mice. By restricting to one gender and thereby avoiding additional variation in our study set up, allows us to better design the group size in our tumor models by taking all the 3R's into account.
However, as suggested by you (the CCD) it can be argued to do the experiments in both sexes, since also cancer appears in both males and females. By just doing this in several tumor models, we can learn from this and try to get more insight if there is a difference between the genders with respect to translation to human studies, as is discussed in the article mentioned before as well.

Considering all this, we want to follow the CCD's suggestions and intend to establish in the coming 5 years several tumor models (3-5) in male mouse strains, some of which are in our laboratory already well characterized in female mice. Once established, we aim to compare the efficacy of several therapeutic antibodies in both sexes to learn about the possible (or lack of) differences with respect to antibody efficacy

We would like to incorporate these additional studies in to the procedures that we applied for. The design is comparable, only male mice are added as a variation. We do not want to mix the genders in one experiment, because we do not want to introduce additional variations within a single study since this can affect the scientific design and outcome of the studies significantly. Furthermore, both sexes in one study have influence on housing and randomization of the experiment and will complicate the design in an unnecessary way. To study the difference in sexes we should run same studies in parallel, one with male and one with female mice.
So, if the CCD wants us to perform a "duplicate" experiment we are open to setup at least 2 or 3 tumor models in male and female mice and explore potential differences in the results in these tumor models.

In animal procedures 3.4.4.1 and 3.4.4.2 we added the following sentence in B :
If the CCD approves, we are open to setup some tumor models in male mice as well and compare the sex difference with respect to antibody efficacy.

7) Bijlage 3 4.4.3 beschrijft oefenen van technieken en in bijlagen 3.4.4.1 en 3.4.4.2 wordt beschreven dat er jaarlijks nieuwe modellen worden ontwikkeld.
Het onderscheid in modelontwikkeling en oefenen van technieken lijkt in de beschrijving een beetje door elkaar te lopen.
Als bijlage 3.4.4.3 alleen om training van de technieken gaat, is het niet helder waarom in bijlage 3.4.4.3 gekozen kan worden voor het in leven laten van de dieren tot 16 weken, met potentieel ernstig ongerief. Wanneer het enkel gaat om oefenen van technieken zouden de dieren ook eerder kunnen worden gedood. Zoals het nu beschreven staat lijkt dit dus ook op modelontwikkeling. Om een en ander te verhelderen zou u, indien nodig, ervoor kunnen kiezen om alle modelontwikkeling en training in bijlage 3.4.4.3 te beschrijven.

We prefer to keep the 'practicing of animal procedures' separately described in 3.4.4.3, since the purpose is most different compared to the other appendices and it also includes only 3-5 animal per technician, which makes the scientific design of a practicing procedure very different.

With respect to the establishment of tumor models, this procedure is very comparable to the procedures described in 3.4.4.1 and 3.4.4.2. The only difference is the antibody treatment and the statistical analysis, which is not included in the establishment of the tumor models, for the rest it is the same. Therefore, we like to keep the description of 'tumor model establishments' in 3.4.4.1 and 3.4.4.2. We also indicated the handlings of the establishments of the tumor model in Table 2. Hopefully, this provides more insight in the handlings and leads to better understanding that it fits best in appendix 1 and 2.

The longer time period indicated in the practicing procedures of 3.4.4.3, had the purpose to check if the outgrowth of the tumor occurs in the right organ/location. When validating this we know that not only the handlings went well, but also the way of tumor cell injection in the correct organ.

We do, however, agree with the CCD that practicing of the handlings technique is the main purpose of this appendix and that observation up to 16 weeks in not necessary. In general, we will observe a tumor development within 1 month, Therefore, observation up to maximal 1 month, should be feasible to check the location of the tumor growth.
Therefore, we adjusted in 3.4.4.3 the '16 week period' to 1 month,

Since we will observe a shorter time period, it is not likely that the animals will reach the humane endpoint related to tumor growth and, therefore, mainly clinical signs will be in focus to check if abortion cristeria are reached. Therefore, we adjusted part of the text in "J":

"We intend to stop most animals in this procedure directly after recovery of surgery. However, in some cases, if recovery is succesfull, animals will be euthanized as soon as we detect tumor appearance. If we observe tumor development, the location of the tumor will be checked and the animals will be euthanized. The maximum observation time is 1 month, if no tumor development is observed, the animals will be euthanized earlier.
Because of this rather short tumor procedure it is not likely that the animals will reach the humane endpoint related to tumor growth.
Humane endpoints in this procedure will therefore mostly be defined by clinical signs and body weight loss as described in point 1 and 2. However we cannot fully predict the timing of tumor take appearance, and since small tumors can also cause adverse effects we might encouter tumor growth related discomfort as well."

Leges
De leges die u verschuldigd bent zijn nog niet door ons ontvangen of de betaling is nog niet verwerkt. Uw aanvraag is niet compleet als de leges niet zijn ontvangen.
Zonder deze aanvullende informatie kan de beslissing nadelig voor u uitvallen omdat de gegevens onvolledig of onduidelijk zijn.

We have checked the status of payment at our financial department and they indicated that the payment was performed on 12 April 2017. So, if correct, you should have it now available on your bank account. If you still have not received the payment, please, inform us, so we can check if something went wrong.

Centrale Commissie Dierproeven

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'.	24400
1.2	Provide the name of the licenced establishment.	Genmab
1.3	Provide the title of the project.	Research and Evaluation of therapeutic test antibodies in tumor models in mice and rats

2 Categories

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

- X Basic research
- X Translational or applied research
- ☐ Regulatory use or routine production
- ☐ Research into environmental protection in the interest of human or
- ☐ Research aimed at preserving the species subjected to procedures
- X 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.

At our company we concentrate on the generation of new recombinant antibodies, and new innovative antibody formats for the treatment of cancer. We continuously invent and generate potential new antibodies and test these pre-clinically as well as evaluate those antibodies in clinical studies.

Cancer is one of the leading causes of death in Western countries including the Netherlands. Many therapies are applied to combat this disease, such as radiation therapy, chemotherapy and immuno-therapy. However, the complexity and heterogeneity of cancer contribute to the fact that not all patients respond to the therapy, or that the cancer returns after a number of years.

The last decade, immunotherapy is an expanding field in the treatment of cancer patients. It is a type of cancer treatment designed to boost the body's natural defenses to fight the cancer. It uses substances either made by the body or in a laboratory to improve or restore immune system function. For example recombinant antibodies for therapeutical use in patients with cancer and/or chronic inflammatory diseases have been proven to be very successful (Scott AM, Nature reviews, 2012).
Recently, the concept of immunotherapy is even appointed as the breakthrough in the treatment of cancer (E Goldin, Nature medicine 2013; J cousin Frankel, Science 2013), indicating the rapid movement of this field.

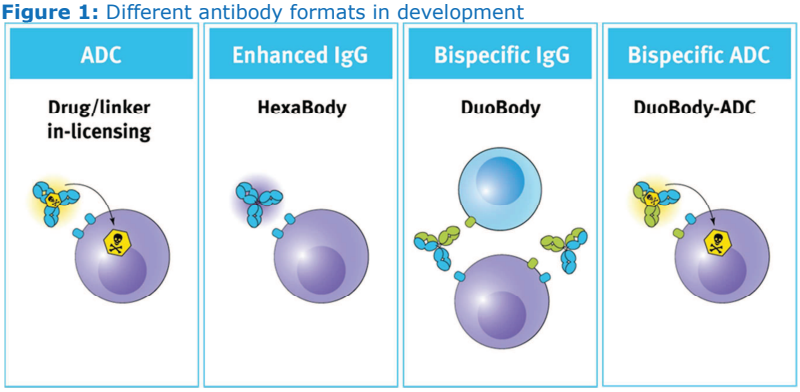
However, because of the heterogeneous characteristics of cancer, still a large number of cancer patients have to face the recurrence of cancer and thus improvement is still required. Though, according to the AACR Cancer progress Report 2016, several new medical products for the treatment of cancer have been approved by the FDA in the last years, it is expected that in the United States in 2016 nearly 600.000 patients will die from some form of cancer (Cancer Statistics 2016, Siegel RL et al, Ca Cancer J Clin, 2016). Therefore, development of new and modified improved antibody formats is a continuous urgent need to further enhance the efficacy and/or safety of these antibody drugs in patients.

At our company we yearly evaluate, in a multidisciplinary team of scientific experts, a large number of novel potential cancer targets that are of interest for antibody targeting therapy. This is done by means of literature search, competitor landscape analysis, collaborations and in vitro studies. Of these targets only a few targets will be selected to start the generation of antibodies for. We have the objective to go forward with only the most promising cancer targets where we see the potential to create new innovative antibody formats for.

The final goal is to generate differentiated antibody therapeutics that are more potent and/or safer than antibodies/drugs currently in the clinic, and have potential to be best-in-class or first-in-class. These criteria will be taken along during the whole antibody selection procedure.
The number of new antibody targets taken into antibody development will differ per year, but will in general be 10-30 per year. This will be done with or without external collaborations.

The antibody formats to be applied to the cancer targets that are designed thus far, and currently in the development by our company are: 'Antibody Drug Conjugates' (ADCs), were a drug/toxin is coupled with a linker to the antibody; Enhanced IgG formats, where enhanced immune effector functions are established; bispecific IgG antibodies, where a dual targeting can be established; and 'bispecific-ADCs', bispecific antibodies with a drug linker (figure 1).

Figure 1: Different antibody formats in development



The following pre-clinical process includes intensive in vitro testing, such as binding characteristics and functional characteristics of the antibody that leads to the kill of the tumor cells or inhibition of tumor cell growth. Besides these initial in vitro processes needed for the first step of antibody characterization, in

vivo efficacy testing in tumor models and pharmacokinetic analysis in mice and rats are inevitable, since only animal studies reflect the complexity as seen in humans/patients.

Recent studies have revealed that new antibody formats can indeed be more potent for the treatment of some cancers compared to (antibody-) drugs already in the clinic. For example, antibody-drug conjugates (ADC), can be more effective in the inhibition of the tumor growth compared to the non-conjugated antibody, as has been demonstrated with Herceptin-DM1 which has been shown to be very effective in breast cancer patients [redacted] Also our own preclinical findings show complete regression of several implanted human tumors, without significant side effects when using ADCs. [redacted] Furthermore, there are strong indications that the use of other new antibody formats developed at our company, such as bispecific antibodies (DuoBody) or HexaBody, can strengthen the potency and specificity of antibodies for the treatment of cancer [redacted]

The research described in this project application focusses on the selection, research and evaluation of antibodies in tumor models in mice and rats and will be a direct continuation of our current research program ("Development of antibodies for therapeutic use: Research and evaluation on subcutaneous tumor models, optical imaging and imaging of subcutaneous tumor models in rodents). By performing these in vivo experiments we aim to get insight in

- the antibody drug efficacy in a "full body system"
- the mechanism of action of the antibody drug in vivo
- optimal dosing of the antibody drug
- Timing and location of activity of the antibody drug
- The tumor type/indication most suitable for the application of the antibody drugs

To explore this, we require a diversity of specific in vivo tumor models to allow the analysis and evaluation of the different antibody-drugs. The specific models will be set up and designed, based on the antibody format, the molecule and the cancer type that is targeted by the respective antibodies.

Parallel to the studies described in this project application, we are studying related processes which are part of a successful antibody generation, like immunizations in rodents and pharmacokinetic studies. These related processes are described in two different parallel project applications: "Investigation of pharmacokinetics of antibody formats in rodents" and "Immunization of rodents (transgene for human IgG)".

In short: selection, research and evaluation of the efficacy of new antibodies or antibody formats in a diversity of specific in vivo tumor models is very important for the development of therapeutic antibodies and will give us insight into the mechanisms of actions of these drugs. This will help us to translate and predict the potency of these antibody drugs in cancer patients much better and will help us in the design of clinical trials leading to an improved benefit for cancer patients in the future.

3.2 Purpose

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

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

The main objective of the study described in this application is selection, analysis and evaluation of therapeutic antibodies by evaluating anti-tumor efficacy in a diversity of in vivo tumor models in mice and rats.
The need for such studies is elementary for moving the best antibody candidate forward and bringing a new medicine successfully to the clinic as we have done for several antibodies (such as an anti-CD20 antibody (Arzerra®), for the treatment of chronic leucocyte leukemia, and for an anti-CD38 (Darzalex®), for the treatment of multiple myeloma).

3.3 Relevance

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

Evaluation and research of new antibodies drug candidates or new antibody formats in mice or rat tumor models will give insight in the mode of action of the drugs.
Furthermore, by analyzing the different animal tumor models described in this application, we will gain understanding in the use of these different tumor models in antibody therapy and the translatability into cancer patients. Each tumor model type will have its own characteristics, advantages and disadvantages and by studying this (for examples by taking out the tumor and explore the target expression or the immunological cell content) we will gain insight into these models, which will help to refine the design of future tumor models and clinical trials for cancer patients.
Scientific relevant findings will be communicated to collaborators and/or published at specific conferences and/or in scientific magazines.

3.4 Research strategy

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

Before in vivo studies will start, an intensive selection process is performed on a large antibody panel by means of in vitro assays determining the specific target binding and functional activity. Only a small number of the best antibodies will move forward and will be selected for further in vivo research, such as anti-tumor efficacy studies.

Several in vivo animal tumor model studies are needed in different stages of the development process of therapeutic antibodies to address different research questions:

[redacted]

Depending on the molecule and the cancer type that the antibody targets and the mechanism of action of the antibody format (ADC, DuoBody, HexaBody, or others) a diversity of specific in vivo tumor models will be designed, set up and evaluated as is described in 3.4.2 and in both appendices animal procedures.

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.

All models described in this project application are animal tumor models.
Based on the general outline of the experimental design of each individual animal experiment, the tumor model described in this application is divided in 2 different animal procedures.

The majority of tumor model experiments [redacted] will be performed as the animal procedure: "subcutaneous tumor models". The remainders of the experiments that will be performed fall under the second animal procedure: "orthotopic tumor and metastasis models".

A short description of the two animal procedures is described below. A detailed description can be found in the two independent appendices of the animal procedures:

1) Animal procedure 1: Subcutaneous tumor models:
   Subcutaneous injection of tumor cells/fragments in mice or rats followed by tumor volume measurements by Caliper and optional with imaging techniques*

2) Animal procedure 2: Orthotopic tumor (injection of tumor cells at the organ of the cell's origin) and metastasis models:

Different ways of tumor cell injection will be applied in this procedure:

a) Orthotopic tumor cell inoculation without surgery:
Injection of tumor cells in easy accessible organs such as in the mammary gland (subcutaneously into the fat pads), or dermis (intradermally)

b) Orthotopic tumor cell inoculation with surgery:
Direct injection of tumor cells in respective organs after opening of the animal's body, (such as pancreas, ovarium, prostate, etc.), or indirect injection (in some incidences) into organs (such as intra-splenic or portal vein injection (imaging* and/or scoring of tumor loci and/or resection and weighing of tumor in the liver).

c) Intravenous tumor cell inoculation: Injection of tumor cells into the tail vein. This injection method does not need surgery or anesthesia.

d) Intracardiac tumor cell inoculation: Injection of tumor cells into the left ventricle of the heart.

e) Intraperitoneal tumor cell inoculation: Injection of tumor cells into the peritoneal cavity.

In this procedure, tumor development will be mainly followed by imaging* and/or alternatively by caliper measurement and/or scoring of tumor loci and/or resection and weighing of tumor.

*Imaging techniques can include: bioluminescence or fluorescence, radioactivity, CT/PET/SPECT in combination with labeled cells or antibodies or tracers/proteins.

In all these animal procedures three different types of models can be selected based on the antibody target and antibody format:

- Xenograft models: in which mostly human tumor cells/fragments will be implanted in mice or rats.
- Syngeneic models: in which the tumor to be injected as cells or fragments, as well as the host are from the same species/strain (mouse or rat)
- Humanized models: In which the immune system of the mouse or rat is "humanized", such as:
  - Engraftment models with human peripheral blood mononuclear cells (PBMCs)
  - CD34+ Hematopoietic Stem cell (HSC) reconstituted humanized
  - Genetically engineered mouse (and rat) models (GEMMS)

Based on the research strategy, antibody target and antibody format, the most optimal model will be selected.

3) Animal procedure 3: Practicing of techniques for Orthotopic tumor and metastasis models:
In case of new models have to be established for the orthotopic tumor and metastasis models that include surgery or intracardiac injection, we expect the need for practicing to optimize the succes rate of the model establishment and antibody efficacy testing in these tumor models.

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.

We make selections of the animal procedures and models described in paragraph 3.4.2 mainly dependent on the antibody target and on the cancer type where the tumor antigen is expressed (see in figure 2).

Fig 2a: [redacted]

Fig 2b: [redacted]

The outcome of the animal tumor model studies will give us insight and knowledge on the activity and mechanism of action of the drug and will be an important step in the decision making process. Based on this, decisions will be made to either progress the antibody drug candidates forward in the development process, or to be put on hold.
Furthermore, these animal tumor model experiments are very important in the clinical trial design with respect to indication/cancer type selection, dosing and the possibility of biomarkers analysis.

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	Subcutaneous tumor models
2	Orthotopic tumor and metastasis models
3	Practising of techniques for orthotopic tumor and metastasis models
4	
5	
6	
7	
8	
9	
10	

Centrale Commissie Dierproeven

12.

Appendix
Description animal procedures

1. This appendix should be enclosed with the project proposal for animal procedures.
2. A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
3. For more information, see our website (www.centralecommissiedierproeven.nl).
4. 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'.	24400
1.2	Provide the name of the licenced establishment.	[redacted]
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: 1 — Type of animal procedure: 1. Subcutaneous tumor models

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

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

The here described animal procedure includes the **subcutaneous implantation** of human or murine tumor cells mainly, or in some cases patient derived tumor material from approved sources (like CRO's) in:

1. Xenograft models (human tumor cells/fragments in mice or rats),
2. Syngeneic models (murine tumor cells/fragments in mice or rats), or
3. Humanized models (human tumor cells/fragments in mice or rats), mice harboring part of the human immune system (HIS). Examples of HIS mouse are:
   - Subcutaneous co-engraftment with human immune cells (f.e. human PBMCs (Peripheral Blood Mononuclear Cells))
   - intraperitoneal or intravenous engraftment with human immune cells (f.e. PBMCs)
   - $CD34^+$ Hematopoietic stem cell (HSC) reconstitution in young immunocompromised mice. Mice will then be purchased as fully humanized animals.
   - Human Transgenic models (mice will be purchased from appropriate sources)

[redacted]

Secondary parameters may include blood, tissue or tumor analysis. In some models these secondary parameters can be regarded as primary readout parameters, for example in vivo tumor models where circulating blood biomarker analysis is important in relation to the antibody drug, or in tumor models, were the effect of the antibody on the tumor microenvironment is an important mechanism of action. In these experiments tumor tissue collection and analysis will be used as primary readout parameter. But in all these studies tumor volumes will be measured.

In case when new subcutaneous tumor models have to be set up, only the tumor development is addressed with respect to tumor take, growth rate and variation. The purpose of these establishment studies is the determination of the tumor cell inoculation conditions. [redacted]. Procedure will be the same as describe below, except for the antibody treatment.

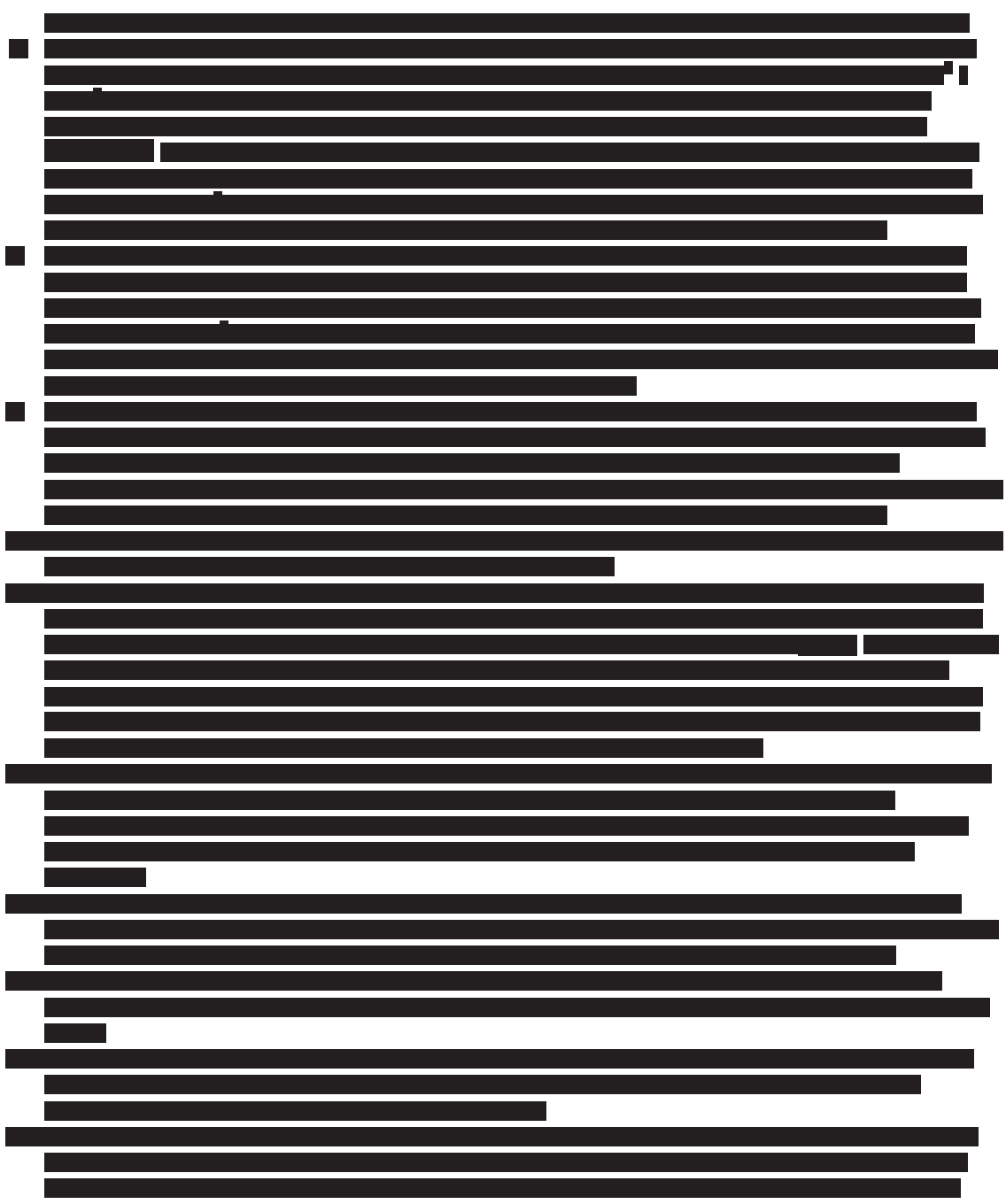
Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

For each animal experiment described in this animal procedure: "subcutaneous tumor models", the following steps will be taken:

- Each individual animal experiment will be discussed, designed and described by a team of experts and will be supported with a rational, study design, group sizes, protocol, statistical analysis and animal welfare expectations.
- All designed animal experiments will be in line with the Code of Practice "Animal experiments in cancer research". Inspectie W&V, (Netherlands Inspectorate for Health Protection, Commodities and Veterinary Public Health), 1999, Zutphen
- Approval of each animal experiment needs to be requested and approved by the AWB and the animal facility.

The general procedure for the subcutaneous tumor models will be as follow

- [redacted]



* total blood sampling will not exceed the maximal limit. Methods for blood sampling include; submandibular vein puncture, vena sephena puncture, tail vein puncture or alternative approved/advised methods.

A general experiment will take normally up to 16 weeks. However, depending on the efficacy of the drug, some animals can be studied for long term resistance or development of immune memory. These studies can take maximally up to 52 weeks.

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

To get an indication for the proper group sizes per study a power/sample size analysis has been done based on the tumor volumes, since the research described in this application indicates "tumor volume" as the primary parameter for tumor burden, a continuous variable readout, calculated on the basis of measurements with (digital) calipers in two dimensions.

Power and groups size analysis:

Group size selection:
Based on the power analysis described above we want to select the following group sizes:

[redacted]

In the group size selection, the scientific outcome and the total number of animal is always taken into consideration.

In all models where antibody treatment is applied and tumor volume will be the primary readout, the relative T/C (tumor volumes of treated animals over control animals values) and "tumor growth inhibition" (TGI) values will be calculated.

Statistical analysis

In general and if possible, a standard statistical analysis will be as much as possible applied as follows:

[redacted]

This analysis can be adapted in the future when new insights appear.

In case of only establishing a new subcutaneous tumor model statistical analysis is not applied, since treatment is absent in these studies.

B. The animals

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

We expect to have an average of [redacted] antibody projects active per year, for which we perform in total an

average [redacted] experiments per year. This includes establishment and efficacy testing studies in both animals procedures described in animal procedure I: subcutaneous tumor models as well as the animals experiments described in animal procedure II: orthotopic tumor and metastasis models. (see also Addenda overview table 1 animals)

Within this animal procedure I (s.c.tumor models) we expect to perform [redacted] experiments per year [redacted] models for efficacy testing [redacted] models to be established).
In average we expect to use [redacted] animals per efficacy testing experiments for the s.c. tumor models, and [redacted] animals per new model to be established [redacted] animals per year.
Establishment of new subcutaneous tumor models in general requires less animal per experiment [redacted] experiment) we expect the development [redacted] models per year. Which is [redacted] animals per year.

This will lead to the final use within this animal procedure I to a total of [redacted] Of this it is anticipated to need [redacted] in the coming 5 years.

There is extensive experience in tumor models in mice and rats which allows translation of the potency of a novel drug to human patients.
For the animal studies described in this application we intend to use different mouse or rat strains

In most of our studies we aim to test antibodies specifically targeting (human) tumor antigens, where human tumor cell lines (or fragments) need to be used for implantation. In order to prevent rejection of the tumor cells, it is necessary to use immune deficient mice and rats. We most frequently use CB17 SCID or nude mice. Both, nude and CB17 SCID mice, lack the T-cells subset allowing a better tumor outgrowth. CB17 SCID mice, in addition, have no B-cells meaning they also have no mouse IgG. For the rats we mostly use athymic nude rats.

Immune competent mice (mostly BALB/c or C57bl/6) will be used for syngeneic tumor models, where it is important to study the tumor microenvironment. At this moment it is well known that the tumor microenvironment is important for the development of the tumor, and by targeting this environment (for example with a specific antibody) it can lead to tumor growth inhibition. In these cases it is important that the antibody used also recognizes the murine targets. Depending on the origin of the murine tumor cell line the right strain for the respective model needs to be chosen.

NOD SCID mice lack T-cells in addition to B-cells, and also functional macrophages, complement components, as well as functional Natural Killer cells. Therefore, these mice are often used to study bispecific antibodies in which one part is directed against the human target on the tumor cell and the other part is focused on the human immune cell. In these mice, human immune cells will be co-transplanted subcutaneously together with human tumor cells. That allows the efficacy testing of a bispecific antibody targeting the human immune cells and the human tumor cells in an in vivo setting as described in (Labrijn et al, PNAS, 2013). For these studies even mice with additional immune deficiencies such as NSG/NOG or BRGS mice can be used as well.

The use of NSG or NOG (NOD-SCID IL-2Rg$^{-/-}$), but also BRGS mice and NOD SCID mice are described for experiments in which human blood cells are intraperitoneally or intravenously implanted. All these mice are immune deficient, lack B-, T- and NK-cells, and have defects in macrophages. When PBMCs are inoculated in these mice, the human T-cells are the most expanding human cell population and can be used for T-cell targeting drugs, which is nicely shown by Bacac M *et.al*. (Clin Can Res, 2016)
The NSG (and the BRGS mice) or derivatives hereof are used for the already mentioned CD34+cell HSC-His model allowing the expansion of a diversity of human immune cells in these mice (Legrand, PNAS, 2011). These Human Immune System (HIS)-mice will be used in experiments were the tumor microenvironment needs to be studied or when a mechanism of action of the drug includes activation or inhibition of the human immune cells or the tumor microenvironment (Morton et al, Cancer Res 2016).

Rat xenograft models using athymic nude rats are mostly used for experiments (e.g. in biomarker

studies) when repetitive blood samples are required in amounts impossible to get from single mice. Athymic nude rats do not have T cells and allows the outgrowth of implanted human tumor cells, although in many cases a pre-treatment with cyclophoshamide or irradiation might be needed for a proper tumor outgrowth with an acceptable variation. In addition, for the evaluation of novel antibody formats rat models might be of interest because the compliment system in rats allows a better ex vivo analysis since the complement components seem much more stable.

We have a strong preference for the use of female mice, because it has been shown that male mice have a different activity in the innate complement system than female mice (Beurskens F et al, Clin Exp Biol, 1999) and this may influence the effectiveness of the antibodies. To minimize the variation within a single test and between different tests (intra- and inter-variation) we prefer to be limited to one gender. This allows a more accurate statistical analysis and can lead to the use of fewer mice per group. In addition, we prefer females because these are easier to accommodate (less mutual aggression) than males and are easier to regroup. We want to avoid to house animals alone in one cage because of aggressiveness, which is often the case with males.
However, we do include in some experiments a mixture of males and females, for example the HIS mice are both male and female since these animals are seen as individuals and will be analyzed on a more individual basis. Furthermore, sometimes we have experiments with males only, for example when we want to study the efficacy of our antibodies in prostate cancer.
If the CCD approves, we are open to setup some tumor models in male mice as well and compare the sex difference with respect to antibody efficacy

In general we use mice at an age between 5 and 14 weeks old. Young mice normally have a much better tumor take rate. Per experiment we try to keep the mice in an age difference of maximal 2 weeks, since a higher difference might lead to difference in tumor outgrowth. However, sometimes we are restricted to a certain age of the mice because of technical feasibilities. For example humanized NSG or BRGS mice are somewhat older before tumor cell injection, since first the human immune system has to be established, which can take up to 12-14 weeks.

For each animal experiment it will be carefully considered which background, strain, gender and age is required for having an optimal or most translatable readout for the research question to be addressed in that particular experiment and the efficacy of an antibody drug.

In the future other or new mice and rat strains might be developed that allow better engraftment of tumor cells and/or humane immune cells, and might be used in the animal studies as well.

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.

Most mice/rats will not be re-used.
However in some experiments we may consider to use animals for training purposes or follow up experiments, before the animals will be euthanized

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

xNo

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

D. Replacement, reduction, refinement

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

Replacement,
In vitro studies to analyze the binding properties of the antibodies to the tumor target and to test their

functional anti-tumor activity will always be performed before the start of any in vivo experiment. This allows a first scaling down step of the antibody panel. However in vitro data only will not give an exclusive and complete insight into the anti-tumor efficacy of the antibody of interest. Testing the efficacy of antibodies in in vivo tumor models represents a more complex system which is more comparable to the tumor of the patient and allows the influence of additional factors, such as blood supply, the tumor microenvironment and the contribution of the immune system to the efficacy of the antibody drug. This results in a better and more complete overview on the efficacy of the drugs and allows a better translation to the use in cancer patients.

Reduction,
All the individual tumor models are carefully designed. The variation of the tumor growth (homogeneous or heterogeneous), the predicted efficacy of the drug, and the research question is always taken into account. At each experiment a well thought experimental design is made in which a good scientific outcome is balanced to the total number of mice used in the experiment. This is supported by a power analysis and suggested group sizes described in 2A.
Furthermore bio-distribution of the antibody of interest to the site of the tumor is also very important for the final efficacy of the drug and can only be determined in in vivo settings. To support this feature the pharmacokinetics of the antibody drugs is studied using a parallel project application which in general uses less mice then a tumor model experiment. Using these data we can detect very well the half-life of the specific antibody in circulation. And together with in vitro data we can better predict the effective dose in the tumor models described in this application. Overall following this strategy, this will lead to the use of fewer mice.

Furthermore, we are intensively involved in setting up alternative model systems such as [redacted] In the coming years new techniques will be developed and evaluated if these techniques can successfully replace, refine or reduce the use of animal experiments. However, to explore this, in the first coming years we expect the need of several animal tumor models tumor to evaluate the applicability of the alternative model systems

Refinement
We put a lot of effort in the refinement of the animal tumor models. By having dedicated people we observe, learn and adjust in consecutive studies, so that we know what to expect and in cases of (unexpected) discomfort we can determine how to act to prevent the animals from reaching the "Human End Point" (HEP).
For the maximal tumor size in mice the code of practise "animal experiments in cancer research" (Inspectie W&V, Zutphen/The Hague 1999) describes a tumor volume of 2000 mm3. In our studies we intend to euthanize the mice when a tumor volume above 1500 mm3 is reached. In this way we reduce the discomfort of the mice due to a large tumor size. Also in case of tumor growth in rats, we will euthanize as much as possible the rats before the suggested HEP of 40 cm3 or a diameter of 4.2 cm in the code of practise.

With respect to the appearance of ulcerations at the site of the tumors our team has made an instruction guide how to act and how to handle in case an open wound is observed. This has been discussed extensively within our Animal Welfare Body and is communicated and instructed to the responsible animal caretakers as well. See Addendum III " tumorgroei gerelateerde verschijnselen")

Finally, we make sure that our dedicated personnel remains skilled and will, therefore, be trained regularly, by participating in courses or to learn new techniques in house. Most handlings described in the project applications are standard techniques, and in cases when new techniques need to be learned we prefer to first practise on dead or terminal animals under anaesthesia.

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

The following measures will be taken to minimize the tumor model related discomfort

1) Intensive observation for clinical signs (like abnormal behaviour, posture and appearance) will be done 1-2 times a week, and increased to 3 times a week in periods were we can expect discomfort.
2) 1 to 3 times a week tumor volumes will be measured and when tumors are above 1500 mm3 the mice will be euthanized, to prevent as much as possible that animals reach the human endpoint of 2000 mm3.
3) In case of models that show a wound at the location of the tumor, observation is intensified or preliminary stopped before severe ulcerations appear.
4) Body weight measurements are included in all experiments were discomfort is expected or when it is unknown.

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?

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

The pain and discomfort induced by the anaesthesia is of higher discomfort then the tumor cell and antibody injection.

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

- In cases were imaging or a terminal hart puncture is applied, the animals will first receive anaesthesia.

I. Other aspects compromising the welfare of the animals

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

In some humanized mice models which are adjusted with humane immune cells, we can expect "Graft versus Host Disease"

Explain why these effects may emerge.

- In these models human PBMCs are injected i.p. or i.v., the human T-cells are the most expanding human cell population. The cytotoxic T-cells then recognize the mouse as non-self and can react with the murine body cells. This graft versus host reaction can be observed by clinical signs such as abnormal behaviour, posture and appearance and can be expected 4-6 week after i.p. or i.v. injection of human PBMCs.
- When the PBMCs are subcutaneous co-injected with the tumor cells, only in a few cases these clinical signs are observed. And a graft versus host reaction can be recognized occasionally in these incidences as well.
- When $CD34^+$ hematopoietic stem cells are implanted in young mice (of 1 -3 weeks old), the human immune cells will be trained in the thymus to recognize the mouse cells as "selves" and, therefore, no graft versus host reaction (or only at a very minimal level) will appear in these mice

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

In the period of expected graft versus host reaction, observation will be increased and if possible mice will be preventively euthanized before reaching this point.

J. Humane endpoints

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

☐ No > Continue with question K.

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

Since tumor cells/fragments are implanted in the mice/rats, we can expect tumor growth-related adverse effects, which can lead to tumor related humane endpoints. We use the following humane endpoints as guidance:

1. A large tumor volume is a specific human endpoint in these studies and will be minitored by caliper measurements troughout the whole study: According to Code of practise animal experiment in cancer research) HEP tumorvolume = 2000 mm3 for mice, and 40 cm^3 or a max diameter of 4.2 cm in rats. However in most cases we stop the mice when tumorvolumes of > 1500 mm^3 are reached
2. Ulcerations at the site of the tumor, are a specific HEP in these studies.
3. Body weight loss (> 20%)
4. Clinical signs such as abnormal behaviour, dyspnea (diffuculties with breathing), salivation, no response on incentives, apathy, involuntary shaking of the body or limbs (tremor), convulsion (where the body muscles contract and relax rapidly and repeatedly), self-mutilation, piloerection, abnormal non-physiological posture, paralysis, severe Diarrhea. The level of severeness of these clinical signs can be used to determine if the humane HEP is reached.
5. Graft versus Host Disease (see I)

When a HEP is reached animals will be taken out of the study and will be prematurely euthanized in most of the cases. In some cases where we observe in an early time-point of the study an (unexpected) adverse event close to HEP we might consider to leave the animals in the study for a very short time (1-3 days) because a repetition of the entire study would consume again several mice. In this case we will increase the observation rate, put them eventually in a separate warmed cage, and/or, when observing weight loss, give a subcutaneous PBS injection to recover body fluid. If after 3 days the animal still is not recovered, the rat/mice will be euthanized

Indicate the likely incidence.

Since we follow the tumor volume frequently, we can anticipate when the HEP with respect to tumor size will be reached. Therefore, only a minimal number of animals will reach the maximal tumor volume as humane endpoint.

In models in which we have intensive experience we do not expect that any of the animals will reach the humane endpoint. However, in models that have heterogeneous tumor growth and/or where we have less experience we can expect more mice to reach the humane endpoint.

Appearance of ulceration at the site of the subcutaneous located tumor is dependent on the tumor cell type, e.g. some tumor have more mucus production, appear therefore more tumid, and can cause irritation of the skin at the site of the tumor more severely than others. For most tumor models we know in advance that ulceration might occur, and we, therefore, can anticipate on the appearance of ulceration. In addition, at the moment when a wound is observed, the monitoring of the whole experiment will be intensified or when it has no impact on the scientific outcome, animals will be euthanized before a severe ulceration appears.

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').

The procedures have in general a moderate level of discomfort. In some cases were no tumor development takes places we can expect only mild discomfort and in some cases when animals reach unexpectedly the HEP the discomfort is higher. In a typical experiment we expect:
In 22% a mild discomfort
In 75% a moderate discomfort
In 3% a severe discomfort

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.

Animals are specifically ordered for this procedure and they cannot be used for another type of experiment since they have been injected with tumor cells

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

13.

Appendix
Description animal procedures

1. This appendix should be enclosed with the project proposal for animal procedures.
2. A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
3. For more information, see our website (www.centralecommissiedierproeven.nl).
4. 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'.	24400
1.2	Provide the name of the licenced establishment.	[redacted]
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 2 Type of animal procedure: 1. Orthotopic tumor and Metastasis models

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

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

The here described animal procedure includes tumor models with orthotopic implantation and other metastasis models of human or murine tumor cells mainly, or in some cases patient derived tumor material from approved sources (like CROs) in:

1. Xenograft models (human tumor cells/fragments in mice or rats),
2. Syngeneic models (murine tumor cells/fragments in mice or rats), or
3. Humanized models (human tumor cells/fragments in mice or rats), mice harboring part of the human immune system (HIS). Examples of HIS mouse are:
   - Subcutaneous co-engraftment with human immune cells (f.e. human PBMCs (Peripheral Blood Mononuclear Cells))
   - intraperitoneal or intravenous engraftment with human immune cells (f.ePBMCs)
   - $CD34^+$ Hematopoietic stem cell (HSC) reconstitution in young immunocompromised mice. Mice will then be purchased as fully humanized animals.
   - Human Transgenic models (mice will be purchased from appropriate sources)

Within this animal procedure different methods for tumor cell inoculation can be used to allow orthotopic tumor growth or metastasis formation as indicated below from a) to e):

a) Orthotopic tumor cell inoculation without surgery:

Breast cancer cell lines can be implanted subcutaneously into the "fat pads" of mice which represent the mammary glands of mice or rats. For melanoma models the tumor cells can be implanted directly intra-dermally. This organ specific injection route can in some cases lead to metastasis in other organs. These implantation methods do not need a surgery involving anesthesia.

b) Orthotopic tumor cell inoculation with surgery:
Cancer models, like those derived from pancreas, prostate, kidney, ovary, colon, or other solid cancer forms, need a surgery to get access to the organ of interest in order to establish successfully an orthotopic tumor model in mice or rats. This organ specific injection route can in some cases lead to metastasis in other distant organs. These implantation methods do need anesthesia and in most cases pain relieving methods as well.

c) Intravenous tumor cell inoculation:
Injection of tumor cells in the bloodstream can lead to metastasis formation in different organs. For leukemic cells this often leads to homing and metastasis in different organs and is also known as "a disseminated tumor model". When intravenous injection in the tail vail is applied for other, mainly "bigger" solid cancer cell, the tumor cells home into the lungs and can be used as a lung metastasis model. This injection method does not need surgery or anesthesia.

d) Intra-cardiac tumor cell inoculation:
Injection of tumor cells into the left heart ventricle can be applied when metastasis to different organs is of interest and when metastasis mainly into the lungs needs to be avoided. This method does not include surgery, but intra-cardiac injection needs the use of anesthesia.

e) Intraperitoneal tumor cell inoculation:
This method can be selected when the influence of the peritoneal microenvironment needs to be studied. In that case for example gastric cancer cells or alternative cell will be implanted intraperitoneally. This injection method does not need surgery or anesthesia.

In general, after tumor cell inoculation, the antibodies of interest (antibody variants, - formats, Antibody Drug Conjugates (ADCs), etc. or combinations hereof) will be administrated with or without a combination of cytostatics or other anti-cancer agents in these models. Tumor growth (in mm3) will be followed in time by imaging (bioluminescence/fluorescence) or alternatively by Caliper measurements, or a combination hereof. "Tumor volume" measured by imaging or by caliper will be the primary readout parameter. Differences in tumor volume, growth rate, tumor growth inhibition or tumor regression induced by the antibody variants compared to the control group and to each other will be addressed in these studies. The outcome will be supported by statistical analysis, where possible, as indicated below.

Secondary parameters may include blood, tissue or tumor analysis. In some models these secondary parameters can be regarded as primary readout parameters. For example in vivo tumor models where circulating blood biomarker analysis is important in relation to the antibody drug. Or in tumor models, were the effect of the antibody on the tumor microenvironment is an important mechanism of action. In these experiments tumor tissue collection and analysis will be used as primary readout parameter. But in all studies tumor volumes will be measured.

In case new orthotopic or metastasis tumor models have to be set up, only the tumor development is addressed with respect to tumor take, growth rate and variation. The purpose of these establishment studies is the determination of the tumor cell inoculation conditions. [redacted] Procedure will be the same as describe below, except for the antibody treatment.

When more complex orthotopic or metastasis models have to be established, such as tumor cell inoculation procedures with surgery or by intracardiac inoculation, we expect the need for practicing upfront establishment of the specific tumor model. This is described in appendix 3.

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

For each animal experiment described in this animal procedure: "orthotopic tumor and metastasis models", the following steps will be taken:

- Each individual animal experiment will be discussed, designed and described by a team of experts. Supported with a rational, study design, group sizes, protocol, statistical analysis and animal welfare expectations.
- All designed animal experiments will be in line with the Code of Practice "Animal experiments in cancer research". Inspectie W&V, (Netherlands Inspectorate for Health Protection, Commodities and Veterinary Public Health), 1999, Zuthpen
- Approval of each animal experiment needs to be requested and approved by the AWB and the animal facility.

The general procedure for orthotopic tumor and metastasis models will be as follow:

[redacted]

[redacted]

[redacted]

* total blood sampling will not exceed the maximal limit. Methods for blood sampling include; submandibular vein puncture, vena sephena puncture, tail vein puncture or alternative approved/advised methods

A general experiment will take normally up to 16 weeks. However, depending on the efficacy of the drug, some animals can be studied for long term resistance, or development of immune memory. These studies can take maximally up to 52 weeks.

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

To get an indication for the proper group sizes per study a power/sample size analysis has been done based on the tumor volumes, since the research described in this application indicates "tumor volume" as the primary parameter for tumor burden, a continuous variable readout, calculated on the basis of measurements with (digital) calipers in two dimensions.

Power and groups size analysis:

[redacted]

[redacted]

Group size selection:
Based on the power analysis described above we want to select the following group sizes:

[redacted]

[redacted]

[redacted]

[redacted]

Statistical analysis

In general and if possible, a standard statistical analysis will be as much as possible applied as follows:

[redacted]

This analysis can be adapted in the future when new insights appear.

In case of only establishing a new orthotopic or metastasis tumor model statistical analysis is not applied, since treatment is absent in these studies

B. The animals

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

We expect to have an average of [redacted] antibody projects active per year, for which we perform in total an average of [redacted] experiments per year. This includes both animals procedures described in animal procedure I: subcutaneous tumor models as well as the animals experiments described in animal procedure II: orthotopic tumor and metastasis models. (see also Addenda Table 1: number of animals)

Within this animal procedure II (orthotopic tumor and metastasis models) we expect to perform [redacted] experiments per year. ([redacted] models for efficacy testing [redacted] models to be established).

In average we expect to use [redacted] animals per experiments for the orthotopic tumor and metastasis models, which will be [redacted] animals per year.

For the establishment of new orthotopic or metastasis tumor models we expect the use of [redacted] animal per experiment. We expect the development of [redacted] models per year. Which is [redacted] animals per year.

This will lead to the final use within this animal procedure to a total of [redacted] animals in 5 years. Of this it is anticipated to need [redacted] in the coming 5 years.

In addition we expect to need of [redacted] newly ordered animals per year for practising of the different techniques described in this project application, which will be [redacted] animals for practicing purposes only for the coming 5 years. We expect the need of [redacted] in total for practicing purposes. This is described in appendix 3: practicing of techniques for orthotopic and metastasis models.

There is extensive experience in tumor models in mice and rats which allows translation of the potency of a novel drug to human patients.
For the animal studies described in this application we intend to use different mouse or rat strains

Especially for orthotopic and metastasis models and/or where imaging is applied we will use mouse and rat strains recommended in the literature for a particular orthotopic/metastasis model, or will use animal strains we have experience with (see below). For syngeneic models we have to use the strains where the cells originated from.

In most of our studies we aim to test antibodies specifically targeting (human) tumor antigens, where human tumor cell lines (or fragments) need to be used for implantation. In order to prevent rejection of the tumor cells, it is necessary to use immune deficient mice and rats. We most frequently use CB17 SCID or nude mice. Both, nude and CB17 SCID mice, lack the T-cells subset allowing a better tumor outgrowth. CB17 SCID mice, in addition, have no B-cells meaning they also have no mouse IgG. For the rats we mostly use A-thymic nude rats.

Immune competent mice (mostly BALB/c or C57bl/6) will be used for syngeneic tumor models, where it is important to study the tumor microenvironment. At this moment it is well known that the tumor microenvironment is important for the development of the tumor, and by targeting this environment (for example with a specific antibody) it can lead to tumor growth inhibition. In these cases it is important that the antibody used also recognizes the murine targets. Depending on the origin of the murine tumor cell line the right strain for the respective model needs to be chosen.

NOD SCID mice lack T-cells in addition to B-cells, and also functional macrophages, complement components, as well as functional Natural Killer cells. Therefore, these mice are often used to study bispecific antibodies in which one part is directed against the human target on the tumor cell and the other part is focused on the human immune cell. In these mice, human immune cells will be co-transplanted together with human tumor cells. That allows the efficacy testing of a bispecific antibody targeting the human immune cells and the human tumor cells in an in vivo setting as described in (Labrijn et al, PNAS, 2013). For these studies even mice with additional immune deficiencies such as NSG/NOG or BRGS mice can be used as well.

The use of NSG or NOG (NOD-SCID IL-2Rg$^{-/-}$), but also BRGS mice and NOD SCID mice are described for experiments in which human blood cells are intraperitoneally or intravenously implanted. All these mice are immune deficient, lack B-, T- and NK-cells, and have defects in macrophages. When PBMCs are inoculated in these mice, the human T-cells are the most expanding human cell population and can be used for T-cell targeting drugs, which is nicely shown by Bacac M *et.al*. (Clin Can Res, 2016)
The NSG (and the BRGS mice) or derivatives hereof are used for the already mentioned CD34+cell HSC-His model allowing the expansion of a diversity of human immune cells in these mice [Legrand, PNAS, 2011). These human immune system (HIS)-mice will be used in experiments were the tumor microenvironment needs to be studied or when a mechanism of action of the drug includes activation or inhibition of the human immune cells or the tumor microenvironment (Morton et al, Cancer Res 2016).

Rat xenograft models using athymic nude rats are mostly used for experiments (e.g. in biomarker studies) when repetitive blood samples are required in amounts impossible to get from single mice. Athymic nude rats do not have T cells and allows the outgrowth of implanted human tumor cells, although in many cases a pre-treatment with cyclophoshamide or irradiation might be needed for a proper tumor outgrowth with an acceptable variation. In addition, for the evaluation of novel antibody formats rat models might be of interest because the compliment system in rats allows a better ex vivo analysis since the complement components seem much more stable.

We have a strong preference for the use of female mice, because it has been shown that male mice have a different activity in the innate complement system than female mice (Beurskens F et al, Clin Exp Biol, 1999) and this may influence the effectiveness of the antibodies. To minimize the variation within a single test and between different tests (intra- and inter-variation) we prefer to be limited to one gender. This allows a more accurate statistical analysis and can lead to the use of fewer mice per group. In

addition, we prefer females because these are easier to accommodate (less mutual aggression) than males and are easier to regroup. We want to avoid to house animals alone in one cage because of aggressiveness, which is often the case with males.
However, we do include in some experiments a mixture of males and females, for example the HIS mice are both male and female since these animals are seen as individuals and will be analyzed on a more individual basis. Furthermore, sometimes we have experiments with males only, for example when we want to study the efficacy of our antibodies in prostate cancer.
If the CCD approves, we are open to setup some tumor models in male mice as well and compare the sex difference with respect to antibody efficacy

In general we use mice at an age between 5 and 14 weeks old. Young mice normally have a much better tumor take rate. Per experiment we try to keep the mice in an age difference of maximal 2 weeks, since a higher difference might lead to difference in tumor outgrowth. However, sometimes we are restricted to a certain age of the mice because of technical feasibilities. For example humanized NSG or BRGS mice are somewhat older before tumor cell injection, since first the human immune system has to be established, which can take up to 12-14 weeks.

For each animal experiment it will be carefully considered which background, strain, gender and age is required for having an optimal or most translatable readout for the research question to be addressed in that particular experiment and the efficacy of an antibody drug.

In the future other or new mice and rat strains might be developed that allow better engraftment of tumor cells and/or humane immune cells, and might be used in the animal studies as well.

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.

Most mice/rats will not be re-used.
However in some experiments we may consider to use some animals for training purposes before the animals will be euthanized.

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

xNo

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

D. Replacement, reduction, refinement

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

Replacement,
In vitro studies to analyze the binding properties of the antibodies to the tumor target and to test their functional anti-tumor activity will always be performed before the start of any in vivo experiment. This allows a first scaling down step of the antibody panel. However in vitro data only will not give an exclusive and complete insight into the anti-tumor efficacy of the antibody of interest. Testing the efficacy of antibodies in in vivo tumor models represents a more complex system which is more comparable to the tumor of the patient and allows the influence of additional factors, such as blood supply, the tumor microenvironment and the contribution of the immune system to the efficacy of the antibody drug. This results in a better and more complete overview on the efficacy of the drugs and allows a better translation to the use in cancer patients.

Reduction,
All the individual tumor models are carefully designed. The variation of the tumor growth (homogeneous or heterogeneous), the predicted efficacy of the drug, and the research question is always taken into

account. At each experiment a well thought experimental design is made in which a good scientific outcome is balanced to the total number of mice used in the experiment. This is supported by a power analysis and suggested group sizes described in 2A.
Furthermore bio-distribution of the antibody of interest to the site of the tumor is also very important for the final efficacy of the drug and can only be determined in in vivo settings. To support this feature the pharmacokinetics of the antibody drugs is studied using a parallel project application which in general uses less mice then a tumor model experiment. Using these data we can detect very well the half-life of the specific antibody in circulation. And together with in vitro data we can better predict the effective dose in the tumor models described in this application. Overall following this strategy, this will lead to the use of fewer mice.

Furthermore, we are intensively involved in setting up alternative model systems such as [redacted] In the coming years new techniques will be developed and evaluated if these techniques can successfully replace, refine or reduce the use of animal experiments. However to explore this, in the first coming years we expect the need of several animal tumor models tumor to evaluate the applicability of the alternative model systems

Refinement
We put a lot of effort in the refinement of the animal tumor models. By having dedicated people we observe, learn and adjust in consecutive studies, so that we know what to expect and in cases of (unexpected) discomfort we can determine how to act to prevent the animals from reaching the "Human End Point" (HEP).
For the maximal tumor size in mice the code of practise "animal experiments in cancer research" (Inspectie W&V, Zutphen/The Hague 1999) describes a tumor volume of 2000 mm3. In our studies we intend to euthanize the mice when a tumor volume above 1500 mm3 is reached. In this way we reduce the discomfort of the mice due to a large tumor size. Also in case of tumor growth in rats, we will euthanize as much as possible the rats before the suggested HEP of 40 cm3 or a diameter of 4.2 cm in the code of practise.

In case of orthotopic tumors or metastasis, we will follow in most cases the tumor development by imaging, and can anticipate on the appearance of HEP related with tumor growth and act accordingly to avoid the HEP as much as possible. When performing consecutive studies with orthotopic models we get a feeling on the time lines and the in vivo luciferase activity. We are then even more cautious when animals reach human endpoints.

With respect to the appearance of ulcerations at the site of the tumors our team has made an instruction guide how to act and how to handle in case an open wound is observed. This has been discussed extensively within our Animal Welfare Body and is communicated and instructed to the responsible animal caretakers as well. See Addendum III " tumorgroei gerelateerde verschijnselen")

Finally, we make sure that our dedicated personnel remains skilled and will, therefore, be trained regularly, by participating in courses or to learn new techniques in house. Most handlings described in the project applications are standard techniques, and in cases when new techniques need to be learned we prefer to first practise on dead or terminal animals under anaesthesia. In some cases as additional mice/or rats will be ordered for the use of practising only (as described in Appendix 3).

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

The following measures will be taken to minimize the tumor model related discomfort:

1) Intensive observation for clinical signs (like abnormal behaviour, posture and appearance) will be done 1-2 times a week, and increased to 3 times a week in periods were we can expect discomfort.
2) 1 to 3 times a week tumor volumes will be measured and when tumors are above 1500 mm3 tumors will be stopped, so that only a minimal number of mice reach the human endpoint of 2000 mm3. When imaging is applied to follow tumor growth we can (based on imaging values,

time point in the study and tumor type) anticipate when HEP can be reached. We will act accordingly to minimize the discomfort of the mice and rats as much as possible, with keeping the scientific value as high as possible.

3) In case of models that show a wound at the location of the tumor, observation is intensified or preliminary stopped before severe ulcerations appear
4) Body weight measurements are included in all experiments were discomfort is expected or when it is unknown.

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?

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

For all treatments with antibodies or other test compounds as well as for the implantation into the mammary fat pads (breast cancer models) or intradermally (melanoma models) or intraveneously (leukemic and other metastasis models) no anesthesia or analgesia is required because the distress for the animals is very low.

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

- For all surgeries as well as intracardiac tumor cell injections the animals will receive anesthesia
- To prevent pain, an analgesic is applied 30 minutes-1 hour before the surgery and can be repeated 24 hours after the implantation.

-The painkillers needed for more severe pain, will only be applied after consultation of the veterinarian

I. Other aspects compromising the welfare of the animals

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

In some humanized mice models which are adjusted with humane immune cells, we can expect "Graft versus Host Disease"

Explain why these effects may emerge.

- In these models human PBMCs are injected i.p. or i.v., the human T-cells are the most expanding human cell population. The cytotoxic T-cells then recognize the mouse as non-self and can react with the murine body cells. This graft versus host reaction can be observed by clinical signs such as abnormal behaviour, posture and appearance and can be expected 4-6 week after i.p. or i.v. injection of human PBMCs.
- When the PBMCs are subcutaneous co-injected with the tumor cells, only in a few cases these clinical signs are observed. And a graft versus host reaction can be recognized occasionally in these incidences as well.
- When $CD34^+$ hematopoietic stem cells are implanted in in young mice (of 1 -3 weeks old),, the human immune cells will be trained in the thymus to recognize the mouse cells as "selves" and, therefore, no graft versus host reaction (or only at a very minimal level) will appear in these mice

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

In the period of expected graft versus host reaction, observation will be increased and if possible mice will be preventively euthanized before reaching this point.

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.

Since tumor cells/fragments are implanted in the mice/rats, we can expect tumor growth related adverse effects, which can lead to tumor related human endpoints. We use the following human endpoints as guidance:

1. A large (visible) tumor volume is a specific human endpoint in these studies and will be minitored by caliper measurements for the breast cancer and melanoma models troughout the whole study: According to Code of practise animal experiment in cancer research) HEP tumor volume = 2000 mm^3 for mice and 40 cm^3 or a max diameter of 4.2 cm in rats. However in most cases we stop the mice when tumorvolumes of > 1500 mm^3 are reached
2. When tumors are not visible, imaging is applied in most studies to follow tumor growth. Based on imaging values, time point in the study and tumor type, we anticipate when the HEP can be reached. Human endpoint is for these non-visible tumors mostly observed by the clinical signs and body weight loss.
3. Ulcerations at the site of the tumor in breast cancer and melanoma orthotopic models have to be considered as a specific HEP in these studies.
4. Body weight loss (> 20%)
5. Clinical signs, most important for orthotopical models since mostly no primary tumor is visible, are: abnormal behaviour, dyspnea (diffuclties with breathing), salivation, no response on incentives, apathy, involuntary shaking of the body or limbs (tremor), convulsion (where the body muscles contract and relax rapidly and repeatedly), self-mutilation, piloerection, abnormal non-physiological posture, paralysis, severe Diarrhea. The level of severeness of these clinical signs can be used to determine if human HEP is reached.
6. For some specific leukemic/metastasis models hind leg paralysis is described as a clinical observation. The tumor cells home to different organs/tissues. The paralysis of the hind legs just prior to death is associated with the presence of neoplastic nodules within the spinal canal. (Jin-Song Yan, et al, Chin J of Cancer 2009) and will be considered as a HEP in these studies.
7. Graft versus Host disease. (this will be only expected in humanized mice, as described in I)

When a HEP is reached animals will be taken out of the study and prematurely euthanized in most of the

cases. In some cases where we observe in an early time-point of the study an (unexpected) adverse event close to HEP we might consider to leave the animals in the study for a very short time (1-3 days) because a repetition of the entire study would consume again several mice. In this case we will increase the observation rate, put them eventually in a separate warmed cage, and/or, when observing weight loss, give a subcutaneous PBS injection to recover body fluid. If after 3 days the animals still is not recovered, the rat/mice has to be euthanized.

Indicate the likely incidence.

Especially for the orthotopic models where we can apply caliper measurements (breast cancer and melanoma models), we can anticipate when the HEP with respect to tumor size will be reached. Therefore, only a minimal of animals will reach the max tumor volume as human endpoint.
For orthotopic and metastasis models, where no primary tumor is easily visible, we visualize primary tumors as well as metastases by applying imaging techniques. In that respect we get a quantitative readout for the tumor and metastasis burden. This, but also the timing in the model and the appearance of clinical signs have to be taken into account to address animal's discomfort and HEP.
In models in which we have intensive experience we do not expect that any of the animals will reach the human endpoint. However, in models that have heterogeneous tumor growth and/or where we have less experience we can expect more mice to reach the human endpoint.

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').

The procedures have in general a moderate level of discomfort. In some cases were no tumor development takes plays we can expect only mild discomfort and in some cases when animals reach unexpectedly the HEP the discomfort is higher. In a typical experiment we expect:
In 10% a mild discomfort
In 87% a moderate discomfort
In 3% a severe discomfort

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.

Animals are specifically ordered for this procedure and they cannot be used for another type of experiment since they have been injected with tumor cells

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

14.

Appendix
Description animal procedures

1. This appendix should be enclosed with the project proposal for animal procedures.
2. A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
3. For more information, see our website (www.centralecommissiedierproeven.nl).
4. 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'.	24400
1.2	Provide the name of the licenced establishment.	[redacted]
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 3 Type of animal procedure: 1. Practicing of several techniques for orthotopic tumor and metastasis tumor models

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

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

The here described animal procedures include only the practicing of different techniques needed to establish orthotopic and/or metastasis models.

We expect the need of additional mice and/or rats for practicing of two procedures described in appendix 2 (orthotopic/metastasis models). These methods include method b) orthotopic tumor cell inoculation with surgery and method d) intracardiac inoculation of tumor cells.
Since we have limited experience with both these techniques, it is important to validate the correct way of tumor cell injection and surgery before establishment and performing antibody efficacy studies. To practice these procedures we will apply standardized techniques that are described in literature or we will be trained by people having experience in the establishment of these models.

Additional animals for practicing are not needed for the following methods: a) orthotopic implantation without surgery, c) intravenous inoculation and e) intraperitoneal inoculation (described in Appendix 2). These techniques are already established and intensively applied in the efficacy testing of the antibodies in development, so no additional practicing is needed. Also for the methods described in appendix 1 (subcutaneous implantation of tumor cells) additional mice /or rats for practicing are not required since this technique is applied in most of our experiments and personnel is already trained and skilled in these techniques.

The parameters in these practising sessions are the successful conduct of proper tumor cell injection techniques in the different organs after surgery (prostate, pancreas, ovarian etc.) or intra-cardially. In addition it is important to monitor the recovery of the animal from the surgery/tumor cell inoculation under anaesthesia and to monitor successful tumor growth.

Typically in training sessions only tumor cells will be inoculated and no antibodies. However, not the tumor take rate or tumor volume will be a primary endpoint but rather the clinical conditions of the mice/rat a few days after the practiced technique/surgery, or tumor cell inoculation. If successfully recovered from surgery, validation of outgrowth of the tumor within the intended organ(s) will be evaluated and imaging of tumor volume can be applied in some of these mice or rats.

In most cases animals will be euthanized a few days after training the tumor cell inoculation. However in cases when animals recover successfully after tumor cell inoculation, it can be considered to follow tumor growth and body weight measurements. This has only the purpose to validate the implantation technique.

These studies do not need the support of statistical analysis.

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

Practicing the different techniques described in this animal procedure: "Practising of several techniques for orthotopic tumor and metastasis models" will be comparable to the technique b) and d) as described in Appendix II.
Before initiation of each practicing session, the following steps will be and taken:

- The animal experiment will be discussed, designed and described by a team of experts. Supported with a rational, protocol, and animal welfare expectations.
- All designed animal experiments will be in line with the Code of Practice "Animal experiments in cancer research". Inspectie W&V, (Netherlands Inspectorate for Health Protection, Commodities and Veterinary Public Health), 1999, Zuthpen
- Approval of each animal experiment needs to be requested and approved by the AWB and the animal facility.

The general procedure for practicing of techniques for orthotopic tumor and metastasis models will be as follow:

[redacted]

After surgery the recovery of the mice/rat will be monitored.

A general practicing procedure will take up to 1 week.
However, in case of successful recovery of the animal after surgery it can be considered to include the steps as described above to monitor the tumor outgrowth. This will allow us to check the specific sites/organs of tumor outgrowth and allow us to validate if the implantation techniques were properly performed. Depending on the tumor growth these can take up to a maximum of 1 month.

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

The animals used in this appendix are only used for practicing purposes, and no statistical analysis will be needed to support the primary endpoints of these practicing studies.)

B. The animals

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

The number of animals needed in these practising procedures will be limited to the number of personnel to be trained ([redacted] per technician). Basic trainings steps (such as localization of the specific organs, orientation and route of injection will be first trained (if possible) on animals euthanized in parallel studies.
For the practising procedures described in this appendix we expect the need of 1 session per year. We foresee the need for practicing these skills for [redacted] technicians, So we expect the need of [redacted] newly ordered animals per year, which will be [redacted] animals for practicing purposes only for the coming 5 years. We expect the need of [redacted] in total. (See also addendum Table 1)

There is extensive experience in tumor models in mice and rats which allows the most optimal tumor growth and translation of the potency of a novel drug to human patients. The mice or rat strain used for these training purposes will be based on the animal strain needed for the particular tumor model to be established.

Details for rational of choice of different rat or mice strains are described in Appendix 2.B.

C. Re-use

Will the animals be re-used?

X No, continue with question D.

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

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

xNo

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

D. Replacement, reduction, refinement

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

The animal studies described in this appendix are used to optimize and refine the animal experiments described in appendix II (orthotopic tumor and metastasis models). By applying these practising procedures before the real establishment of the tumor model, we will enhance the success rate of the new orthotopic tumor and metastasis models.

By performing these practising studies we make sure that our dedicated personnel remains skilled. Therefore, training on a regular basis, either by participating in courses or by learning new techniques in house is essential. Also for these new techniques, specific training or dedicated external courses will increase the skills on these techniques

Furthermore the 3R's described in appendix I and II also apply for these practising studies.

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

The following measures will be taken to minimize the tumor model related discomfort:

1) The training techniques will be performed under anaesthesia, and if needed painkillers will be applied.
2) Intensive observation for clinical signs (like abnormal behaviour, posture and appearance) will be done the first days after surgery/tumor cell inoculation.

3) Only when recovery of the mice/rat is successful by means of visual clinical signs (like normal behaviour, motility, absence of piloerection), follow up monitoring can be initiated in some of the mice or rats. If so,
   - body weight measurements are included.
   - When using luciferase or fluorescent tumor cells tumor volumes will be monitored up to weekly by imaging. At the moment that tumors can be visualized by imaging, it can be validated if the tumor growth occurs in the expected organ(s). When this is confirmed, the animal will be euthanized.

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.

- For all surgeries as well as intracardiac tumor cell injections the animals will receive anesthesia
- To prevent pain, an analgesic is applied 30 minutes-1 hour before the surgery and can be repeated 24 hours after the implantation.

-The painkillers needed for more severe pain, will only be applied after consultation of the veterinarian

I. Other aspects compromising the welfare of the animals

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

NA

Explain why these effects may emerge.

-

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

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.

Since this appendix includes only practising of new techniques, it might be that mice or rats encounter discomfort/stress/complications after surgery due to the moderate skills on these certain techniques by the technician(s). However, before starting practising on the animals in this protocol, ex vivo training will be performed first by means of theoretical training and training on sacrificed animals in parallel projects.

We intend to stop most animals in this procedure directly after recovery of surgery. However, in some cases, if recovery is succesfull, animals will be euthanized as soon as we detect tumor appearance. If we observe tumor development, the location of the tumor will be checked and the animals will be euthanized. The maximum observation time is 1 month, if no tumor development is observed, the animals will be euthanized earlier.
Because of this rather short tumor procedure it is not likely that the animals will reach the humane endpoint related to tumor growth.
Humane endpoints in this procedure will therefore mostly be defined by clinical signs and body weight loss as described in point 1 and 2. However we cannot fully predict the timing of tumor take appearance, and since small tumors can also cause adverse effects we might encouter tumor growth related discomfort as well.

1. Clinical signs, most important for orthotopical models since mostly no primary tumor is visible, are: abnormal behaviour, dyspnea (diffuclties with breathing), salivation, no response on incentives, apathy, involuntary shaking of the body or limbs (tremor), convulsion (where the body muscles contract and relax rapidly and repeatedly), self-mutilation, piloerection, abnormal non-physiological posture, paralysis, severe Diarrhea. The level of severeness of these clinical signs can be used to determine if humane endpoint is reached.
2. If we observe longer than 1 week, bodyweight measurements will be included. At Body weight loss (> 20%) the humane endpoint is reached.

When a Humane endpoint is reached animals will be directly euthanized.

Indicate the likely incidence.

K. Classification of severity of procedures

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

The procedures in general cause a moderate level of discomfort. In some cases where we euthanize the animals under anaesthesia we expect only mild discomfort. However in most cases we will monitor the recovery of the animals from anaesthesia and follow the mice/rats to check the tumor formation after 1 to 3 weeks. In some cases where the animals do not successfully recover from surgery severe discomfort can be encountered. Therefore in general we expect in the practising procedures:
In 10% mild discomfort

In 84% moderate discomfort
In 6% severe discomfort

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.

Animals are specifically ordered for this procedure and they cannot be used for another type of experiment since they have been injected with tumor cells

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

17.

Centrale Commissie Dierproeven

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Aanvraagnummer
AVD2440020171264
Bijlagen
1

25 APR 2017

Datum 24 april 2017
Betreft Beslissing aanvraag projectvergunning Dierproeven

Geachte mevrouw [redacted],

Op 31 maart 2017 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Research and Evalution of therapeutic test antibodies in tumor models in mice and rats" met aanvraagnummer AVD2440020171264. Wij hebben uw aanvraag beoordeeld.

Op 20 april 2017 heeft u uw aanvraag aangevuld. Naar aanleiding van onze vragen is de NTS aangepast naar eenvoudiger taal op enkele punten, is het ongerief van de dieren duidelijker beschreven in de NTS, is de doelcategorie aangevuld, is hergebruik correct ingevuld, is de acclimatisatieperiode verlengd van 3 dagen naar 1 week, zijn de handelingen bij de dieren en de reden voor doden van de dieren verhelderd, het gebruik van beide geslachten beschreven, en de experimentduur van de handelingen in bijlage 3.4.4.3 verkort.

Beslissing
Wij keuren uw aanvraag goed op grond van artikel 10a van de Wet op de Dierproeven (hierna: de wet). Hierbij gelden de voorwaarden zoals genoemd in de vergunning.
De algemene voorwaarde(n) zijn opgenomen op grond van artikel 1d lid 4, artikel 10a1 lid 2, artikel 10 lid 2 en/of artikel 10a3 van de wet.
Vanwege het translationele karakter van het onderzoek, en om het aantal dieren in voorraad gedood te beperken, wordt een voorwaarde toegevoegd m.b.t. het gebruik van beide geslachten.

U kunt met uw project "Research and Evalution of therapeutic test antibodies in tumor models in mice and rats" starten. De vergunning wordt afgegeven van 1 mei 2017 tot en met 1 mei 2022.

Overige wettelijke bepalingen blijven van kracht.

Beoordeling achteraf
Na afloop van het project zal er een beoordeling plaatsvinden, zoals bedoeld in artikel 10a1, lid 1d en lid 3, in de wet. Meer informatie over de eisen bij een beoordeling achteraf vindt u in de bijlage.
Beoordeling achteraf dient plaats te vinden vanwege ernstig ongerief bij een deel van de dieren.

Procedure
Bij uw aanvraag heeft u een advies van de Dierexperimentencommissie Utrecht gevoegd. Dit advies is opgesteld op 29 maart 2017. Bij de beoordeling van uw aanvraag is dit advies betrokken overeenkomstig artikel 10a, lid 3 van de wet.
In aanvulling op het DEC-advies stelt de CCD voorwaarden. De voorwaarden staan in de vergunning beschreven. Voor het overige nemen wij het advies van de DEC over, inclusief de daaraan ten grondslag liggende motivering.
Het DEC-advies en de in de bijlage opgenomen beschrijving van de artikelen van de wet- en regelgeving zijn de grondslag van dit besluit.

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

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

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

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

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

Datum:
24 april 2017
Aanvraagnummer:
AVD2440020171264

Centrale Commissie Dierproeven
namens deze:

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:	Genmab B.V.
Adres:	Yalelaan 60
Postcode en plaats:	3584 CM UTRECHT
Deelnemersnummer:	24400

deze projectvergunning voor het tijdvak 1 mei 2017 tot en met 1 mei 2022, voor het project "Research and Evalution of therapeutic test antibodies in tumor models in mice and rats" met aanvraagnummer AVD2440020171264, volgens advies van Dierexperimentencommissie Utrecht. Hierbij is afgeweken van het DEC-advies. Er worden aanvullende voorwaarde(n) gesteld.
De functie van de verantwoordelijk onderzoeker is [redacted]
De aanvraag omvat de volgende bescheiden:

1 een aanvraagformulier projectvergunning dierproeven, ontvangen op 31 maart 2017
2 de bij het aanvraagformulier behorende bijlagen:
a Projectvoorstel, zoals ontvangen per digitale indiening op 20 april 2017;
b Niet-technische Samenvatting van het project, zoals ontvangen per digitale indiening op 20 april 2017;
c Advies van dierexperimentencommissie d.d. 29 maart 2017, ontvangen op 31 maart 2017.
d De aanvullingen op uw aanvraag, ontvangen op 20 april 2017

Aanvraagnummer:
AVD2440020171264

Naam proef	Diersoort/ Stam	Aantal dieren	Ernst	Opmerkingen
3.4.4.1 Subcutaneous tumor models				
	Muizen (Mus musculus) /	[redacted]	3% Ernstig 75% Matig 22% Licht	
	Ratten (Rattus norvegicus) /	[redacted]	3% Ernstig 75% Matig 22% Licht	
3.4.4.2 Orthotopic tumor and Metastasis models				
	Muizen (Mus musculus) /	[redacted]	3% Ernstig 87% Matig 10% Licht	
	Ratten (Rattus norvegicus) /	[redacted]	3% Ernstig 87% Matig 10% Licht	
3.4.4.3 Practicing of several techniques for orthotopic tumor and metastasis tumor models				

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	Muizen (Mus musculus) /	[redacted]	6% Ernstig 84% Matig 10% Licht	
	Ratten (Rattus norvegicus) /	[redacted]	6% Ernstig 84% Matig 10% Licht	

Voorwaarden

Op grond van artikel 10a1 lid 2 van de Wet op de dierproeven zijn aan een projectvergunning voorwaarden te stellen
In dit project worden dierproeven toegepast die vallen in de categorie ernstig volgens artikel 10b van de wet en wordt daarom voorzien van beoordeling achteraf.Deze beoordeling zal uiterlijk april 2023 plaatsvinden. Er zal dan beoordeeld worden of de doelstellingen van het project werden bereikt. Daarnaast wordt bekeken of de schade die de dieren hebben ondervonden, het aantal en soorten proefdieren en de ernst de dierproeven conform de vergunning waren.

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.

Tenminste 2 tumormodellen zullen worden opgezet in zowel mannelijke als vrouwelijke muizen en verschillen in de resultaten hierin zullen worden onderzocht.

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De CCD ziet graag aan het einde van de looptijd van de vergunning een terugkoppeling van de ervaringen opgedaan met het opzetten van de modellen in beide geslachten. Dit kan ook geïncorporeerd in een nieuwe aanvraag.

Centrale Commissie Dierproeven

Aanvraagnummer:
AVD2440020171264

Weergave wet- en regelgeving

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

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

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

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

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

Beoordeling achteraf
Volgens artikel 10a1, lid 1d en lid 3 van de wet worden projecten waarbij niet-menselijke primaten worden gebruikt, projecten die als ernstig ingedeelde dierproeven omvatten of een dierproef die leidt tot ernstige mate van pijn, lijden, angst of blijvende schade die waarschijnlijk langdurig zal zijn en niet kan worden verzacht, achteraf beoordeeld worden.